FUNBIO1
FUNCTIONAL BIOLOGY

BOOK OF ABSTRACTS

1st International Conference on Functional Biology

International House of Scientists “Frédéric Joliot-Curie”
10 - 12 September 2025
Varna, Bulgaria

INSTITUTE OF PLANT PHYSIOLOGY AND GENETICS IPPG
1869 BULGARIAN ACADEMY OF SCIENCES
UNION OF SCIENTISTS IN BULGARIA

FIRST INTERNATIONAL CONFERENCE ON FUNCTIONAL BIOLOGY

10 – 12 September 2025

International House of Scientists “Frédéric Joliot-Curie”, St. Constantine and Helena Resort, Varna, Bulgaria

FOREWORD

The First International Conference on Functional Biology is organised by the Institute of Plant Physiology and Genetics at the Bulgarian Academy of Sciences in collaboration with the Section “Plant Physiology and Biochemistry” of the Union of Scientists in Bulgaria.

The conference marks the 15th anniversary of the Institute of Plant Physiology and Genetics, established through the merger of two prestigious research institutions – the Institute of Plant Physiology “Acad. Metodiy Popov” and the Institute of Genetics “Acad. Doncho Kostov”.

Today, the Institute of Plant Physiology and Genetics is recognized as a national center for fundamental and applied science research with strengths in plant biology. The Institute advances functional biology by the integration of plant physiology, genetics and molecular biology, supported by interdisciplinary approaches and active national and international collaborations.

This conference will present research aligned with the main scientific directions of IPPG: Molecular Biology and Genetics, Plant Ecophysiology and Experimental Algology, and also encourage dialogue with related disciplines. It aims to bring together researchers and experts to share recent advances in functional biology and explore its diverse, cross-disciplinary applications. Contributions extending beyond plant science are welcome with the goal to foster new collaborations and expand the boundaries of integrative biological research.

ORGANIZING COMMITTEE

Prof. Dr. Valya Vassileva – Conference Chairperson – Institute of Plant Physiology and Genetics, BAS

Prof. Dr. Katya Georgieva – Institute of Plant Physiology and Genetics, BAS

Prof. Dr. Iskren Sergiev – Institute of Plant Physiology and Genetics, BAS

Assoc. Prof. Dr. Dessislava Todorova – Institute of Plant Physiology and Genetics, BAS

Assoc. Prof. Dr. Gergana Mihailova – Institute of Plant Physiology and Genetics, BAS

Assist. Prof. Dr. Ivan Iliev – Institute of Plant Physiology and Genetics, BAS

Assist. Prof. Dr. Elena Shopova – Institute of Plant Physiology and Genetics, BAS

Assist. Prof. Dr. Irina Boycheva – Institute of Plant Physiology and Genetics, BAS

Assist. Prof. Dr. Mariyana Georgieva – Institute of Plant Physiology and Genetics, BAS

MSc Tsvetina Nikolova – Institute of Plant Physiology and Genetics, BAS

Mrs Neli Naydenova – Institute of Plant Physiology and Genetics, BAS

SCIENTIFIC COMMITTEE

Prof. DSc Nina Atanassova – Institute of Experimental Morphology, Pathology and Anthropology with Museum, BAS

Prof. Dr. Svetlana Misheva – Institute of Plant Physiology and Genetics, BAS

Assoc. Prof. Dr. Kalina Ananieva – Institute of Plant Physiology and Genetics, BAS

Assoc. Prof. Dr. Lyudmila Simova-Stoilova – Institute of Plant Physiology and Genetics, BAS

Assoc. Prof. Dr. Maria Geneva – Institute of Plant Physiology and Genetics, BAS

Assoc. Prof. Dr. Irina Vaseva – Institute of Plant Physiology and Genetics, BAS

Assoc. Prof. Dr. Georgi Bonchev – Institute of Plant Physiology and Genetics, BAS

Assoc. Prof. Dr. Ina Aneva – Institute of Biodiversity and Ecosystem Research, BAS

Assoc. Prof. Dr. Miroslava Zhiponova – Sofia University “St. Kl. Ohridski”

Prof. Dr. Alma Balestrazzi – University of Pavia, Italy

Prof. Dr. Federico Martinelli – University of Florence, Italy

Prof. Dr. Kamil Růžička – Institute of Experimental Botany of the Czech Academy of Sciences

Prof. Dr. Sigita Jurkonienė – Nature Research Centre, Vilnius, Lithuania

Prof. Dr. Yalçın Kaya – Trakya University, Edirne, Türkiye

CONFERENCE TOPICS

PLANT PHYSIOLOGY AND BIOCHEMISTRY
Physiological and biochemical mechanisms in plants under normal and stress conditions

GENETICS AND EPIGENETICS
From gene regulation to heritable mechanisms driving adaptation and evolution of organisms

CELL AND MOLECULAR BIOLOGY
Cellular structures and molecular networks involved in the regulation of growth, development and stress responses

BIOTECHNOLOGIES FOR SUSTAINABLE DEVELOPMENT
Biotechnological tools and applications for environmental protection, human health and sustainable agriculture

BIODIVERSITY AND GENETIC RESOURCES
Conservation and use of biological diversity to maintain ecological balance and food security

OUR KEY SPEAKERS

Valya Vassileva
Institute of Plant Physiology and Genetics, Bulgarian Academy of Sciences (Bulgaria)
Planting Ideas, Growing Knowledge – 15 Years of Functional Biology at the Institute of Plant Physiology and Genetics

Pernilla Lavesson
Competence Attraction & Professional Integration Lead, Media Evolution, Malmö (Sweden)
Crossing borders: creative collaboration models for scientific innovation

Kamil Růžička
Institute of Experimental Botany, Czech Academy of Sciences (Czech Republic)
Regulation of Hypocotyl Bending by Alternative Splicing. Seeking for Instructions from Above

Andreas Börner
Leibniz Institute of Plant Genetics and Crop Plant Research (Germany)
Plant Genetic Resources – The Pre-Requisite for Future Breeding and Research

Petar Lambrev
HUN-REN Biological Research Centre, Szeged (Hungary)
Functional Diversity of Photosynthetic Supercomplexes on the Ultrafast Timescale

Alexander G. Ivanov
Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Sciences (Bulgaria)
Phenotypic Plasticity of Overwintering Plant Species: Maximizing Energy Conversion Efficiency and Seed Yield

Ivelin Rizov
Directorate Rural Development, Ministry of Agriculture and Food (Bulgaria)
Regulation of Modern Biotechnologies in the European Union: Challenges and Prospects for their Sustainable Application

OUR SPONSORS

CONFERENCE PROGRAM

10 September 2025 (Wednesday)			
13:00 - 15:00	Registration		
15:00 - 15:40	Opening Ceremony	Diana Petkova	Welcome address from the Union of Scientists in Bulgaria.
		Katya Georgieva	Greetings and welcome from the Section "Plant Physiology and Biochemistry" – Union of Scientists in Bulgaria.
		Valya Vassileva	Planting ideas, growing knowledge – 15 years of functional biology at the institute of plant physiology and genetics.
Evening Session: 15:40 - 17:00			
15:40 - 16:10	Plenary Lecture	Pernilla Lavesson	Crossing borders: creative collaboration models for scientific innovation.
16:10 - 16:40	Oral Presentation	Georgi Bonchev	DNA barcoding and metabarcoding technologies for sustainable solutions to societal needs – a Bulgarian perspective.
16:40 - 17:00	Oral Presentation	Volodymyr Strashnyuk	Variability in polyteny in the salivary glands of the fruit fly *Drosophila melanogaster* (Diptera: Drosophilidae): is there a relationship with fitness?
17:00 - 17:10	Sponsor Presentation	Omnimed Ltd.	Medical equipment and reagents.
19:00	Welcome Cocktail		

Acad. Metodiy Popov

Acad. Doncho Kostov

In 1947, Acad. Doncho Kostov established the Institute of Applied Biology at the Bulgarian Academy of Sciences (BAS).

In 1948, Acad. Metodiy Popov founded the Institute of General Biology at BAS.

Over time, both institutes changed their structure and scientific focus numerous times.

In 1954, Acad. Popov's institute was renamed the Institute of Plant Physiology.

In 1976, during its final transformation, the Institute of Applied Biology became the Institute of Genetics at BAS and was later named after its founder, Acad. Doncho Kostov.

In 2010, the Institute of Plant Physiology and the Institute of Genetics merged into a single institute, which is still known today as the "Institute of Plant Physiology and Genetics."

CONFERENCE PROGRAM

11 September 2025 (Thursday)			
Morning Session: 09:30 - 12:30			
09:30 - 10:00	Plenary Lecture	Andreas Börner	Plant genetic resources – the pre-requisite for future breeding and research.
10:00 - 10:20	Oral Presentation	Svetlana Misheva	Genome-wide association studies for advancing sustainable genetic improvements in bread wheat.
10:20 - 10:40	Oral Presentation	Vasilissa Manova	Investigation on the effect of regenerative organic farming strategies on the productivity of *Solanum tuberosum* and *Phaseolus vulgaris* and soil substrate parameters.
10:40 - 11:20	Coffee Break		
11:20 - 11:50	Plenary Lecture	Kamil Růžička	Regulation of hypocotyl bending by alternative splicing. Seeking for instructions from above.
11:50 - 12:10	Oral Presentation	Emil Vatov	Does cytosine methylation play a role in regulating flowering time under water deficit in *Arabidopsis thaliana*.
12:10 - 12:30	Oral Presentation	Violeta Ivanova	The exocyst tethering complex as a determinant of brassinosteroid receptor trafficking in plants.
12:30 - 14:30	Lunch Break		
Afternoon Session: 14:30 - 18:30			
14:30 - 15:00	Plenary Lecture	Petar Lambrev	Functional diversity of photosynthetic supercomplexes on the ultrafast timescale.
15:00 - 15:20	Oral Presentation	Brigitta Lantos	Mitophagy in resurrection plant *Haberlea rhodopensis*: vacuolar intrusion of mitochondria responds to chilling stress.
15:20 - 15:40	Oral Presentation	Veronika Deleva	Microalgae in sustainable agriculture.
15:40 - 16:20	Coffee Break		
16:20 - 16:50	Plenary Lecture	Alexander G. Ivanov	Phenotypic plasticity of overwintering plant species: maximizing energy conversion efficiency and seed yield.
16:50 - 17:10	Oral Presentation	Jurga Jankauskienė	Physiological and biochemical responses of rapeseed plant under herbicide stress are modulated by pre-application of microbial biostimulant.
17:10 - 18:30	Poster Session		
19:00	Social Dinner		

CONFERENCE PROGRAM

12 September 2025 (Friday)			
Morning Session: 09:30 - 11:00			
09:30 - 10:00	Plenary Lecture	Ivelin Rizov	Regulation of modern biotechnologies in the European Union: challenges and prospects for their sustainable application.
10:00 - 10:20	Oral Presentation	Alan Blanc	Antifungal potential of *Neltuma hassleri* against clinically relevant opportunistic fungi.
10:20 - 10:40	Oral Presentation	Tsvetina Nikolova	Assessment of dose-dependent influence of spermine on the germination of wheat seeds and the mycelial development of pathogenic *Fusarium* fungus.
10:40 - 11:00	Closing Ceremony		
11:00 - 11:30	Coffee Break		
11:30 - 12:30	Poster Session		
12:30 - 14:00	Lunch Break		
14:00 - 17:00	Excursion		

Aladzha Monastery is the most famous medieval cave monastery along the Bulgarian Black Sea coast. It was inhabited by hermit monks during the XIII-XIV centuries.

It was in the end of XIX century when the founders of the Bulgarian archaeology - Shkorpil brothers, Karel and Hermin, began systematically to study this Christian monument. In 1927, Aladzha Monastery was declared a public historical monument and in 1968 it was declared an architectural cultural monument of national significance.

The monastery caves are hewn on two levels into an almost 40 m high limestone rock. The first level consists of a monastery church, monastic cells, a dining premise and a kitchen, a small cemetery church, a crypt (bone-vault) and farm premises. The second level is a natural cave recess in the eastern end of which there is a monastery chapel.

LIST OF PRESENTATIONS

PLENARY LECTURES PL–01-07

PL–01 **Valya Vassileva** – Planting ideas, growing knowledge – 15 years of functional biology at the Institute of Plant Physiology and Genetics.

PL–02 **Pernilla Laveson** – Crossing borders: creative collaboration models for scientific innovation.

PL–03 **Andreas Börner** – Plant genetic resources – the pre-requisite for future breeding and research.

PL–04 **Kamil Růžička, Ivan Kashkan, Viveka** – Regulation of hypocotyl bending by alternative splicing. Seeking for instructions from above.

PL–05 **Petar Lambrev, Parveen Akhtar, Sumit Singhal, Ferenc Sárlos, Hoang-Long Nguyen, Yue Feng, Wenda Wang, Jian-Ren Shen, Howe-Siang Tan** – Functional diversity of photosynthetic supercomplexes on the ultrafast timescale.

PL–06 **Alexander G. Ivanov, Norman P. A. Huner** – Phentypic plasticity of overwintering plant species: maximizing energy conversion efficiency and seed yield.

PL–07 **Ivelin Rizov** – Regulation of modern biotechnologies in the European Union: challenges and prospects for their sustainable application.

ORAL PRESENTATIONS OP–01-11

OP–01 **Georgi Bonchev** – DNA barcoding and metabarcoding technologies for sustainable solutions to societal needs – a Bulgarian perspective.

OP–02 **Volodymyr Strashnyuk, Lyubov Shakina, Daria Skorobagatko** – Variability in polyteny in the salivary glands of the fruit fly *Drosophila melanogaster* (Diptera: Drosophilidae): is there a relationship with fitness?

OP–03 **Svetlana Misheva, Tanya Kartseva, Vladimir Aleksandrov, Ahmad M. Alqudah, Andreas Börner** – Genome-wide association studies for advancing sustainable genetic improvements in bread wheat.

OP–04 **Vasilissa Manova, Dimitar Danchev, Zornitsa Stoyanova, Georgi Bonchev, Miroslava Zhiponova** – Investigation on the effect of regenerative organic farming strategies on the productivity of *Solanum tuberosum* and *Phaseolus vulgaris* and soil substrate parameters.

OP–05 **Emil Vatov, Tsanko Gechev** – Does cytosine methylation play a role in regulating flowering time under water deficit in *Arabidopsis thaliana*.

OP–06 **Violeta Ivanova, Ilina Poparova, Eugenia Russinova, Kiril Mishev** – The exocyst tethering complex as a determinant of brassinosteroid receptor trafficking in plants.

OP–07 **Áron Keresztes, Brigitta Müller, Éva Sárvári, Péter Nyitrai, Hong-Diep Pham, Gergana Mihailova, Gabriella Szalai, László Sas, Katya Georgieva, Imre Vass, Ádám Solti** – Mitophagy in resurrection plant *Haberlea rhodopensis*: vacuolar intrusion of mitochondria responds to chilling stress.

OP–08 **Veronika Deleva, Juliana Ivanova, Nadya Agayn-Stoyanova** – Microalgae in sustainable agriculture.

OP–09 **Jurga Jankauskienė, Rima Mockevičiūtė, Elžbieta Jankovska-Bortkevič, Tautvydas Žalnierius, Sigita Jurkonienė, Zornitsa Katerova, Iskren Sergiev, Dessislava Todorova** – Physiological and biochemical responses of rapeseed plant under herbicide stress are modulated by pre-application of microbial biostimulant.

OP–10 **Alan Blanc, Estefanía Butassi, Ricardo Orquera, Juan Zabala, Maximiliano Sortino, Laura Svetaz** – Antifungal potential of *Neltuma hassleri* against clinically relevant opportunistic fungi.

OP–11 **Tsvetina Nikolova, Dessislava Todorova, Tzenko Vatchev, Zornitsa Stoyanova, Valya Lyubenova, Yordanka Taseva, Ivo Yanashkov, Iskren Sergiev** – Assessment of dose-dependent influence of spermine on the germination of wheat seeds and the mycelial development of pathogenic *Fusarium* fungus.

POSTER PRESENTATIONS PP–01-35

PP–01 **Elisaveta Kirova, Maria Geneva, Maria Petrova, Kamelia Miladinova-Georgieva, Mariana Sichanova, Daniela Tsekova** – Organic nanoparticle-assisted stress modulation in *Stevia rebaudiana*: biochemical impacts of M6 and M6+IAA in *in vitro* cultures.

PP–02 **Liliana Brankova, Tsvetina Nikolova, Elena Shopova, Zornitsa Katerova, Dessislava Todorova, Zornitsa Stoyanova, Iskren Sergiev** – Spermine seed priming mitigates *Fusarium culmorum* stress in wheat.

PP–03 **Radoslav Chipilski, Petar Chavdarov, Irina Moskova, Zoya Tsekova, Elisaveta Kirova, Konstantina Kocheva, Iskren Sergiev** – Response of einkorn and winter wheat plants to treatment with plant growth regulator MEIA and NH_4NO_3 fertilization.

PP–04 **Dessislava Todorova, Tsvetina Nikolova, Yordanka Taseva, Svetoslav Anev, Iskren Sergiev** – The effect of polyamine spermine on key photosynthetic parameters in *Fusarium* infected winter wheat.

PP–05 **Dessislava Todorova, Irina Vaseva, Liliana Brankova, Elena Shopova, Zornitsa Katerova, Iskren Sergiev** – Drought stress effects on enzymatic antioxidants in Bulgarian winter wheat (cv. Fermer) are modulated by melatonin root pre-treatment.

PP–06 **Miroslava Zhiponova, Grigor Zehirov, Krasimir Rusanov, Mila Rusanova, Miroslava Stefanova, Tsveta Ganeva, Momchil Paunov, Valentina Ganeva, Kiril Mishev, Petre I. Dobrev, Roberta Vaculíková, Václav Motyka, Zhenya Yordanova, Ganka Chaneva, Valya Vassileva** – Blue-red led light triggers physiological, phytochemical and structural adaptive responses in the medicinal plant *Nepeta nuda*.

PP–07 **Gergana Mihailova, Ádám Solti, Eva Popova, Liliana Gigova, Katalin Solymosi, Brigitta Lantos, Enkhjin Enkhbileg, Antoaneta V. Popova, Maya Velitchkova, Lyudmila Simova-Stoilova, Katya Georgieva** – Protective mechanisms in roots contributing to the recovery of *Haberlea rhodopensis* from desiccation.

PP–08 **Zornitsa Katerova, Tsvetina Nikolova, Elena Shopova, Liliana Brankova, Dessislava Todorova, Ivo Yanashkov, Iskren Sergiev** – Seed pre-treatment with spermine modulates stress outcome in *Triticum aestivum* L. plants infected with *Fusarium culmorum*.

PP–09 **Simona Galabova, Lyudmila Simova-Stoilova, Heorhii Balzhyk, Tsvetina Nikolova, Asya Petrova, Zornitsa Katerova, Desisslava Todorova, Iskren Sergiev, Thomas Depaepe, Valya Vassileva, Dominique Van Der Straeten, Irina I. Vaseva** – Profiling of antioxidant enzymes-coding genes in drought-stressed *Arabidopsis thaliana* plants with cell type-specific attenuation of ethylene signalling.

PP–10 **Elena Shopova, Zornitsa Katerova, Irina Vaseva, Dessislava Todorova, Iskren Sergiev** – Root application of melatonin modulates non-enzymatic antioxidants and glutathione reductase in wheat subjected to drought stress.

PP–11 **Elena Shopova, Liliana Brankova, Tsvetina Nikolova, Zornitsa Katerova, Dessislava Todorova, Tzenko Vatchev, Iskren Sergiev** – The effect of seed priming with spermine on phenotypical alterations and enzymatic antioxidant defense in *Fusarium*-infected wheat plants.

PP–12 **Iskren Sergiev, Elena Shopova, Liliana Brankova, Irina Vaseva, Zornitsa Katerova, Dessislava Todorova** – Pre-treatment with melatonin modulates enzymatic antioxidants in drought treated winter wheat cv. Gines.

PP–13 **Vladimir Aleksandrov, Violeta Peeva, Aleksandar Rumenov, Dilyana Doneva-Naseva** – Different responses to drought in tolerant and sensitive wheat varieties: a comparative analysis.

PP–14 **Iskren Sergiev, Tsvetina Nikolova, Dessislava Todorova, Valya Lyubenova, Svetoslav Anev** – Spermine priming modulates photosynthesis in *Triticum aestivum* L. grown on soil infected with *Fusarium culmorum* (wm. G. Sm.) Sacc.

PP–15 **Tautvydas Žalnierius, Dominykas Laibakojis, Saulė Rapalytė, Jurga Būdienė, Sigita Jurkonienė** – Genome-wide analysis of gibberellin oxidase genes and hormonal response to GA_3 in *Heracleum sosnowskyi*.

PP–16 **Yordan Georgiev, Zornitsa Tileva, Miroslava Zhiponova, Detelina Petrova** – Effect of light spectrum on the physiological state and bioactive potential of the carnivorous plant *Drosera rotundifolia*.

PP–17 **Tsveta Angelova, Christo Angelov, Nikolai Tyutyundzhiev** – Effect of UV irradiation (single and combined) on model and wild-growing plants.

PP–18 **Emil Vatov, Sintia Aneva, Anna Alexandrova, Mihail Angelov, Avanish Rai, Tsanko Gechev** – Biocrop: genetic and metabolomic insights into seaweed biostimulant priming for stress tolerance and nutritionally superior crops.

PP–19 **Sigita Jurkonienė, Virgilija Gavelienė, Rima Mockevičiūtė, Mariam Zreyan, Jurga Jankauskienė, Elžbieta Jankovska-Bortkevič, Vaidevutis Šveikauskas** – Microbial biostimulant and calcium synergy in improving drought tolerance of winter crops.

PP–20 **Alan Blanc, Lucila Garcia, Joaquín Costa, Catalina Feuli, Kiril Mishev, María Rosa Marano** – Evaluation of the effect of aqueous extracts of *Rosmarinus officinalis* on root growth of A*rabidopsis thaliana* and its possible relationship with auxin signaling.

PP–21 **Zlatina Gospodinova, Georgi Antov, Svetozar Stoichev, Trayana Kamenska, Natalia Krasteva, Miroslava Zhiponova** – *In vitro* cytostatic and proapoptotic properties of aqueous extract from flowers of wild-grown *Nepeta nuda* L. towards cancer cell lines.

PP–22 **Ani Georgieva, Inna Sulikovska, Reneta Toshkova, Tanya Toshkova-Yotova, Margarita Dimitrova, Elena Georgieva, Krasimira Tasheva** – Anticancer potential of *in vitro* cultivated bulgarian medicinal plants.

PP–23 **Mariyana Georgieva-Tsenova, Valya Vassileva** – Promoting biodiversity and sustainability through valorisation of orphan legumes (PROSPER) - big ideas from modest beans.

PP–24 **Reni Kalfin, Lyubka Tancheva, Stefan Panaiotov** – Red blood cells and microbiome biodiversity.

PP–25 **Stela Dragomanova, Lyubka Tancheva, Valentina Gavazova, Silviya Abarova, Valya B. Grigorova, Svetlin Tzonev, Dana Stanciu, Reni Kalfin** – Therapeutic potential of *Satureja montana* derived polyphenols in the central nervous system disorders.

PP–26 **Rumyana Valkova, Plamena Marinova, Stoyanka Gurak, Elena Apostolova- Kuzova, Vesselin Baev, Galina Yahubyan, Mariyana Gozmanova** – Elimination of *Candidatus phytoplasma solani* from *Petunia hybrida* via *in vitro* culture of apical meristems combined with thermotherapy.

PP–27 **Mariyana Gozmanova, Lilyana Nacheva, Rumyana Valkova, Ivaylo Tsvetkov** – Optimizing micropropagation of *Prunus mahaleb* (L.) rootstock in Plantform™ tis bioreactor.

PP–28 **Maria Geneva, Kamelia Miladinova-Georgieva, Mariana Sichanova, Lyudmila Dimitrova, Margarita Dimitrova, Maria Petrova** – Accumulation of antioxidant metabolites in *in vitro* propagated *Arnica montana* with yeast extract and salicylic acid.

PP–29 **Kamelia Miladinova-Georgieva, Maria Geneva, Maria Petrova, Mariana Sichanova, Lyudmila Dimitrova, Margarita Dimitrova** – Changes in biometrics and activity of antioxidant enzymes in *in vitro* cultivated *Arnica montana* after elicitation with methyl jasmonate.

PP–30 **Maria Petrova, Maria Geneva, Kamelia Miladinova-Georgieva, Magdalena Sozoniuk, Kiril Mishev, Mariana Sichanova, Lyudmila Dimitrova, Margarita Dimitrova, Viktoria Ivanova, Antoaneta Trendafilova** – Accumulation of phenolic compounds in *Arnica montana in vitro* plantlets after treatment with methyl jasmonate.

PP–31 **Krasimira Tasheva, Mariya Petrova, Margarita Dimitrova, Ludmila Dimitrova, Elena Georgieva, Ani Georgieva** – *In vitro* cultivation of medicinal plants as a tool for biodiversity conservation and biologically active substances production.

PP–32 **Stiliana Simeonova, Georgi Bonchev, Vesselin Doytchinov** – Metabarcoding honey to assess patterns of honeybee plant foraging preferences along an urbanization and landscape gradient.

PP–33 **Michal Hrabovský, Martina Michalová, Miroslav Švec, Dominika Gahurová, Alexander Milovanov, Andrej Palaj, Irina Boycheva, Georgi Bonchev** – Evolutionary patterns in the genus *Symphyotrichum* in East-Central Europe assessed by DNA barcoding.

PP–34 **Dimitar Danchev, Slavil Peykov, Anton Pozumentshtikov, Momchil Paunov, Ana-Maria Nedelcheva, Desislava Mantovska, Miroslava Zhiponova** – Soil oxidation-reduction potential and microbial biodiversity of mulched *Solanum tuberosum* and *Phaseolus vulgaris*.

PP–35 **Ina Aneva, Petar Zhelev, Evgeni Tsavkov, Nikolay Tsvetanov** – Application of DNA barcoding for studying the taxonomy of Bulgarian white oaks (*Subgenus Quercus*).

PLENARY LECTURES

PL–01

PLANTING IDEAS, GROWING KNOWLEDGE – 15 YEARS OF FUNCTIONAL BIOLOGY AT THE INSTITUTE OF PLANT PHYSIOLOGY AND GENETICS

Valya Vassileva

Department of Molecular Biology and Genetics, Institute of Plant Physiology and Genetics, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria
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Over the past 15 years, the Institute of Plant Physiology and Genetics (IPPG) at the Bulgarian Academy of Sciences has established itself as a national center for functional biology, focused on uncovering the fundamental principles underlying plant life. Formed from the merger of two leading institutes in 2010, IPPG integrates molecular biology, genetics, plant ecophysiology and algal biology to explore how plants function - from gene regulation and cellular signaling to whole-organism responses and ecological interactions. With over 600 peer-reviewed publications and coordination of national and EU-funded projects, including Horizon Europe and COST Actions, the Institute has made valuable contributions to understanding plant development, adaptation and resilience. Notable achievements include pioneering studies in plant epigenetics, the establishment of an algal bioresource platform and the early integration of AI and omics approaches in precision agriculture. IPPG supports a vibrant research community and actively mentors the next generation of scientists through international collaborations and training programs. Looking ahead, the Institute is transitioning to become the Institute of Functional Biology, with a major focus on the “how” and “why” of plant processes - their molecular mechanisms, regulatory dynamics and integrated functions — rather than solely the “what” and “where” of static plant features. By advancing fundamental knowledge and addressing pressing environmental challenges such as climate change and food security, IPPG continues to plant ideas and grow knowledge for a sustainable future.

Keywords: Functional biology; plant molecular biology; gene regulation; plant stress responses; systems biology; epigenetics; synthetic biology; sustainable agriculture.

PL–02

CROSSING BORDERS: CREATIVE COLLABORATION MODELS FOR SCIENTIFIC INNOVATION

Pernilla Lavesson

Competence Attraction & Professional Integration Lead, Media Evolution, Malmö, Sweden
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In a time when global challenges demand fresh ways of thinking, the boundaries between science, technology, design and communication are becoming ever more interconnected, opening new pathways for collaboration and innovation. This presentation will explore how cross-disciplinary industries, and particularly platforms like Media Evolution in Malmö, serve as catalysts for collaboration across traditionally disconnected sectors. Drawing from the vibrant Swedish innovation ecosystem, we will share real-world examples and case studies where researchers, artists, technologists and policymakers have co-created solutions with tangible societal impact. From functional biology to sustainable urban development, these collaborations demonstrate how open networks, participatory design and interdisciplinary spaces can accelerate innovation, increase public understanding of science and create pathways from lab-based discoveries to global change. The presentation will provide practical insights into building inclusive environments and inspire a reimagining of the role of scientists not only as creators of knowledge but as active shapers of innovation landscapes where science and society meet.

Keywords: Interdisciplinarity; innovation ecosystems; functional biology; participatory design, cross-sector partnerships; societal impact

Acknowledgements: Sincere thanks are extended to the Malmö Residency Program for fostering connections and collaborations that inspire and create valuable opportunities for knowledge exchange.

PL–03

PLANT GENETIC RESOURCES – THE PRE-REQUISITE FOR FUTURE BREEDING AND RESEARCH

Andreas Börner

Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany
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Plant genetic resources for food and agriculture (PGRFA) play a major role for global food security. The most significant and widespread mean of conserving PGRFA is ex situ conservation. World-wide 7.4 million accessions are stored in about 1,750 *ex situ* genebanks. Plant *ex situ* genebank collections comprise seed genebanks, field genebanks as well as *in vitro* and cryo collections. Species whose seed can be dried, without damage, down to low moisture contents can be conserved in specially designed cold stores. Such “orthodox” seeds can be expected to maintain a high level of vigour and viability for decades. Field genebanks, *in vitro* and cryo storage are used primarily for species which are either vegetatively propagated or which have non-orthodox seeds that cannot be dried and stored for long periods. With a total inventory of 150,000 accessions from 3,212 plant species and 776 genera, the ‘Federal *ex situ* Genebank of Germany’ in Gatersleben holds one of the most comprehensive collections worldwide. It comprises wild and primitive forms, landraces as well as old and more recent cultivars of mainly cereals but also other crops.

Since the majority of genebank accessions globally are stored in the form of seed, seed longevity is of particular importance for crop germplasm preservation. At the IPK research was initiated for a range of crops stored in the genebank over decades. Variation between crop species was detected. However, there is also intraspecific variation within genebank collections. It was concluded that the differences in germination after long term storage are genetically based. Therefore, genetic analyses of seed longevity were initiated. Genetic mapping was performed for barley, wheat, oilseed rape and tobacco.

Furthermore, in cereals, mainly wheat and barley, a number of bi-parental mapping populations and association mapping panels have been established to allow for the genetic analysis of various traits. The current focus covers resistance/tolerance to a number of biotic and abiotic stresses, in particular drought and cold.

Keywords: Crop plants; Germplasm conservation; Seed longevity; Plant genetics.

PL–04

UPSTREAM CUES UNDERLYING THE ROLE OF ALTERNATIVE SPLICING IN AUXIN-MEDIATED HYPOCOTYL TROPIC RESPONSES

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Alternative splicing (AS) is an important, highly intricate, and relatively underexplored regulatory step in gene expression. Very little is known about the specific pathways regulating particular splice events towards determined developmental outputs. We have previously reported AS of the gene encoding major auxin transporter PIN7 as an intriguing example documenting the impact of AS on the morphogenesis of the model plant *Arabidopsis thaliana*. In this scheme, we demonstrated that AS leads to the production of the more active PIN7a and less active PIN7b isoform that interact on the plasma membrane to tune auxin-mediated tropic response.

In a follow-up project, we aim to identify potential regulators upstream of this AS event. Our candidate approach revealed, among others, (1) a group of spliceosomal components and also (2) factors involved in RNA methylation as physiologically relevant regulators of the *PIN7* splice site choice. All candidate lines show a higher content of the *PIN7a* over the *PIN7b* transcript; also, the levels of the translated PIN7a protein are elevated. Consistently, these lines also display a faster tropic response. The mechanistic aspects underlying these findings will be further discussed.

Keywords: Auxin; tropism; alternative splicing; Arabidopsis.

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PL–05

FUNCTIONAL DIVERSITY OF PHOTOSYNTHETIC SUPERCOMPLEXES ON THE ULTRAFAST TIMESCALE

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Photosynthesis employs an evolutionary conserved biochemical machinery including the photosystem I and photosystem II reaction centres that share a high degree of homology among all oxygenic phototrophs from cyanobacteria to higher plants. Nevertheless, photosynthetic organisms have evolved remarkably diverse light-harvesting antenna systems adapted and tailored to the specific living conditions in each possible habitat. The light-harvesting complexes increase the absorption cross-section of the photosystems and actively and dynamically regulate the excitation flow to the reaction centres balancing efficient energy capture and photoprotection. In recent years, the structures of many different antenna-reaction centre supercomplexes have been determined potentially revealing different strategies and mechanisms ensuring efficient light harvesting [1]. Ultrafast time-resolved spectroscopy, and more specifically two-dimensional electronic spectroscopy (2DES), is a powerful tool to probe the excitation energy transfer from the antenna to the reaction centre [2]. We have investigated the dynamics of excitation energy transfer in several types of multisubunit antenna and antenna-reaction centre supercomplexes isolated from plants [3], cyanobacteria [4] and algae [5], using time-resolved fluorescence spectroscopy and 2DES. A comparison between supercomplexes from plants and the diatom *Thalassiosira pseudonana* revealed markedly different antenna organizations and energy transfer dynamics. Rapid energy transfer on timescales of a few picoseconds was measured between the fucoxanthin-chlorophyll protein (FCP) complexes and both photosystems II and I, suggesting a previously underappreciated functional aspect of the FCPs antenna system. Despite structural and dynamic differences, a universal cross-species strategy for light harvesting is proposed as a blueprint for artificial energy-converting systems.

Keywords: Light harvesting; chlorophyll; time-resolved fluorescence; photosystem I; photosystem II;

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PL–06

PHENOTYPIC PLASTICITY OF OVERWINTERING PLANT SPECIES: MAXIMIZING ENERGY CONVERSION EFFICIENCY AND SEED YIELD

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Daniel Arnon first proposed the notion of a 'grand design of photosynthesis' in 1982 to illustrate the central role of photosynthesis as the primary energy transformer for all life on Earth. This concept can be extended to the broad impact of photosynthesis not only in global energy transformation but also in the regulation of plant growth, development, survival and crop productivity through chloroplast redox signalling. The role of chloroplast redox imbalance, measured as excitation pressure, in governing acclimation to abiotic stress and phenotypic plasticity was compared and contrasted. Although all photoautrophs sense excessive excitation energy through changes in excitation pressure, the response to this chloroplast redox signal is species dependent. Due to a limited capacity to adjust metabolic sinks, cyanobacteria and green algae induce photoprotective mechanisms which dissipate excess excitation energy at a cost of decreased photosynthetic performance. In contrast, terrestrial, cold tolerant plants such as cereals and wheat in particular enhance metabolic sink capacity which leads to enhanced photosynthetic performance and biomass accumulation with minimal dependence on photoprotection. The family of nuclear C-repeat binding transcription factors (CBFs) associated with the frost resistance locus, FR2, neighbouring the vernalization locus,VRN1, and mapped to chromosome 5A of wheat, may be critical components that link leaf chloroplast redox regulation to enhanced photosynthetic performance, the accumulation of growth-active gibberellins and the dwarf phenotype during cold acclimation. We summarize our present knowledge of the regulation of photostasis and photosynthetic performance versus photoprotection in response to cold acclimation/vernalization and conclude that the enhanced photosynthetic performance of winter crops is due to an inherent increase in photosynthetic energy conversion efficiency induced by vernalization which translates into high seed yield in the field as well as under controlled environment conditions. The Cold Binding Factor family of transcription factors (*CBFs/DREBs*) governs enhanced photosynthetic performance of winter crops. In contrast, spring varieties survive cold environments by stimulating photoprotection at the expense of photosynthetic performance like that observed for green algae and cyanobacteria. Consequently, this minimizes the photosynthetic energy conversion efficiency of spring varieties and limits their seed yield upon cold acclimation. Further genetic, molecular and biochemical research to confirm these links and to elucidate the molecular mechanism by which chloroplast redox modulation of CBF expression leads to enhanced photosynthetic performance is required. Because of the superior abiotic stress tolerance of cold tolerant winter wheat and seed yields that historically exceed those of spring wheat by 30–40%, the potential to exploit winter cereals for the maintenance or perhaps even the enhancement of cereal productivity under future climate change scenarios that will be required to feed a growing human population is discussed.

Keywords: Photostasis; energy conversion; CBFs; seed yield.

PL–07

REGULATION OF MODERN BIOTECHNOLOGIES IN THE EUROPEAN UNION: CHALLENGES AND PROSPECTS FOR THEIR SUSTAINABLE APPLICATION

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Modern biotechnologies, including genetically modified organisms (GMOs), new genomic techniques (NGTs), and synthetic biology, offer strategic solutions for sustainable agriculture, precision medicine, and the development of the bioeconomy. The current regulatory framework of the European Union (EU), primarily based on Directive 2001/18/EC and Regulation (EC) No. 1829/2003, does not correspond to scientific progress. The 2018 ruling by the Court of Justice of the EU, which equated organisms developed through NGTs with conventional GMOs, further illustrates the discrepancy between science and regulatory practice.

There are legal, scientific, and societal challenges in the regulation of biotechnology in the EU, with particular attention paid to the risk assessment approach, the distinction between process- and product-oriented strategies, and the need for effective public communication. The Food and Agriculture Organization of the United Nations (FAO) and the Organisation for Economic Co-operation and Development (OECD) play key roles in the global dialogue on modern biotechnologies, promoting sustainable, safe, and ethical applications. They do not automatically equate NGTs with traditional GMOs but emphasize the need for a science-based approach and differentiation between technologies and products. They advocate for a risk-based rather than technology-based regulatory model. In this regard is presented also the European Commission's 2023 proposal for differentiated regulation of plants obtained through NGTs, aiming for better alignment with modern science and the goals of sustainable development.

Artificial Intelligence (AI) plays a key role in the future of biotechnology, as this synergy lays the foundation for a new scientific and technological revolution. This raises questions of transparency, algorithmic accountability, and acceptable limits of genetic intervention. Flexible regulations and ethical frameworks are needed. As a first step, innovations can be tested in a protected environment, in so-called "regulatory sandboxes."

An interdisciplinary approach and the active role of policy and society are essential for the responsible development of these technologies. A science-based, risk-proportionate regulatory framework is needed - one that simultaneously fosters responsible innovation and ensures a high level of safety and ethical standards. The active involvement of the scientific community in policy-making, cross-sectoral evaluation, and public dialogue is a key trigger for the full utilization of modern biotechnologies' potential in support of the Green Deal and the sustainable future of the EU food system.

Keywords: Modern biotechnologies; EU regulation; Genome editing; CRISPR; NGT; Artificial intelligence; Sustainability; GMO legislation; Risk assessment; FAO; OECD.

ORAL PRESENTATIONS

OP–01

DNA BARCODING AND METABARCODING TECHNOLOGIES FOR SUSTAINABLE SOLUTIONS TO SOCIETAL NEEDS – A BULGARIAN PERSPECTIVE

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DNA barcoding is a molecular marker technology for rapid, accurate and cost-effective taxonomic identification, monitoring and open-access cataloguing of biodiversity. The *Barcode of Life Initiative* has expanded beyond the scientific arena and become a practical tool with several applications impacting society in a range of social and economic sectors. By improving our understanding of biodiversity, societies can utilize and manage biological resources more sustainably and responsibly.

Biodiversity knowledge from barcoding can benefit various public sectors and stakeholders: food product authentication; identification and management of pest and invasive species in agriculture and horticulture; control of trade of endangered species; forensic investigations and more. Further, the integration of environmental DNA (eDNA) analysis from water, soil or air samples (containing DNA traces left behind by all species in the respective environment emerges) has emerged as a leading method for assessing the dynamics of biodiversity and species interactions at broader ecological scales. These approaches increasingly support ESG (*Environmental, Social, and Governance*) biodiversity reports helping governments and businesses to integrate biodiversity into sustainability efforts. Strategic actions in this field are essential for conservation and biodiversity restoration. Furthermore, integration of DNA barcoding research with education provides a direct and engaging way to bring 'real-life science' into classrooms, promoting biodiversity awareness and inspiring environmentally responsible future generations.

This presentation provides an overview of the societal applications of DNA barcoding and metabarcoding (including commercial ones) with emphasis on current national efforts to strengthen research capacity and multi-sector collaborations in Bulgaria. The presentation will also highlight achievements and future perspectives for sustainable integration of Bulgaria into the European biodiversity research and policy landscape.

Keywords: DNA molecular markers; Biodiversity; Ecology; Food authentication; Education; Public engagement.

OP–02

VARIABILITY IN POLYTENY IN THE SALIVARY GLANDS OF THE FRUIT FLY *DROSOPHILA MELANOGASTER* (DIPTERA: DROSOPHILIDAE): IS THERE A RELATIONSHIP WITH FITNESS?

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Polyteny is a variety of developmental polyploidy that is widespread in plants and animals. Polytene chromosomes are the result of endocycles, a variant of the cell cycle based on endoreduplication. This phenomenon is the basis of postmitotic growth caused by cell expansion. It also provides increased gene expression in eukaryotic cells. The various biological effects of endopolyploidy are currently the subject of much debate, but its full significance remains unclear. The purpose of this study was to investigate genetic variability of the degree of chromosome polyteny in the salivary glands of *Drosophila melanogaster* (Meigen, 1830) in relation to differential fitness of different genotypes. The material for the study was 16 strains, lines and hybrids of fruit flies. Chromosome differences in polyteny were studied by cytomorphometry. According to the obtained results, cell ploidy patterns, as well as average polyteny levels, are subject to significant hereditary variations. Differences between strains and lines, the influence of inbreeding, chromosome isogenization, hybridization, and adaptively significant selection are revealed. Sex differences and various degrees of individual variability in cell ploidy in different strains, lines, and hybrids are shown. It has been found that genetic distances during outbreeding, inbreeding, or hybridization, which largely determine the selective value of different genotypes, also affect polyteny patterns. This suggests that variations in polyteny correlate with genotypic differences in fitness. The genetic component in the total variability in the level of chromosome polyteny in *Drosophila* salivary glands was 45.3%, the effect of sex was 9.5%. Genetic, epigenetic, and humoral aspects of endocycle regulation, which may underlie variations in the degree of chromosome polyteny, as well as the biological significance of the phenomenon of endopolyploidy, are discussed.

Key words: Polytene chromosomes; endoreduplication; genotype; selective value; sex differences.

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OP–03

GENOME-WIDE ASSOCIATION STUDIES FOR ADVANCING SUSTAINABLE GENETIC IMPROVEMENTS IN BREAD WHEAT

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Bread wheat is one of the world's most important cereal crops, serving as a cornerstone of food security for a significant portion of the global population. Since its domestication 10,000 years ago, wheat has been continuously shaped by human selection. With the rediscovery of Mendel's laws of heredity, a science-driven approach to crop improvement gained momentum, leading to the Green Revolution of the 1960s. Since then, wheat breeding has progressed significantly, supported by high-throughput DNA marker technologies, the release of the wheat reference genome, and the recent integration of advanced techniques such as marker-assisted selection and genomic selection. These tools enable breeders to enhance crop adaptability, grain yield, and quality – critical components for ensuring a sustainable food supply in the face of climate fluctuations and a growing global population. A key element in these techniques is the use of molecular markers. Genome-wide association studies (GWAS) have become powerful tools for revealing relationships between phenotypic traits and underlying genotypes across many crops, including wheat. The markers identified through GWAS facilitate more efficient selection of desirable traits. In this study, we provide a concise introduction to the GWAS approach and share our findings regarding its utility in dissecting key agronomic traits in wheat – specifically, grain protein content (GPC) and thousand kernel weight (TKW), crucial determinants of quality and yield. We assessed the phenotypic variation in GPC and TKW within a panel of 179 modern and traditional Bulgarian cultivars and landraces, as well as GPC variation in a diverse set of 255 accessions from 27 countries. These two panels had previously been genotyped using 25K and 90K iSelect SNP arrays, respectively. GWAS was conducted using state-of-the-art statistical methods (MLM Q+K model and FarmCPU). Our results revealed four novel marker-trait associations for GPC and two for TKW within the Bulgarian collection, and three environmentally stable markers associated with GPC within the diverse panel. The most relevant candidate genes near these loci include those encoding storage proteins, as well as proteins involved in senescence-associated nutrient remobilization, germination-related proteolysis, macromolecules biosynthesis, regulatory functions, and metal ion transport and homeostasis. The availability of molecular markers within or near these candidate genes offers promising opportunities for wheat breeding programs, providing functional markers to accelerate genetic gains in yield and grain quality.

Keywords: Association mapping; candidate genes; grain protein content; grain yield; TKW.

Acknowledgements: This research was funded by the Bulgarian National Science Fund, grant number KP-06-N31/17.

OP–04

INVESTIGATION ON THE EFFECT OF REGENERATIVE ORGANIC FARMING STRATEGIES ON THE PRODUCTIVITY OF *SOLANUM TUBEROSUM* AND *PHASEOLUS VULGARIS* AND SOIL SUBSTRATE PARAMETERS

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Climate change and the depletion of soil resources caused by long-term intensive agricultural practices necessitate the development of innovative approaches to improve soil fertility and ensure the production of healthy, high-quality crops. Integration of organic farming into modern agriculture plays a key role in maintaining the ecological balance, thereby ensuring the sustainable existence of the human population. Regenerative agriculture encompasses a set of practices that stimulate plant growth and protection through the use of biological agents, ensuring the renewal of soil physicochemical parameters and biodiversity, while providing nutrient-rich agricultural production. Mulch application is a classical practice in which a cover of natural or artificial material is placed on the soil surface, aiming to maintain optimal conditions for plant growth.

The present project is designed as a fundamental study on the effect of mulching on soil substrate properties and associated microorganisms, as well as its direct and indirect impact on the physiological state of model plants - potato (*Solanum tuberosum*) and bean (*Phaseolus vulgaris*). Our goal is to investigate the mechanisms that influence plant productivity, the rhizosphere microbiome and the soil substrate after the application of mulch of natural origin in the selected crops. The effect of mulching will be monitored within the framework of a developed multi-year program for regenerative agriculture on selected experimental fields, adding techniques such as the use of cover crops, green manure, minimal tillage, precise monitoring of soil and physiological parameters. The knowledge gained during the study will contribute to clarifying the effect of mulching on model crops, the taxonomic structure of the soil microbiome and the interaction between agricultural crops, soil microbiome and soil substrate in the context of the climatic and geographical conditions in Bulgaria. The application of molecular techniques such as DNA barcoding and metabarcoding will provide information on the taxonomic diversity of neighboring organisms in the different experimental setups, which will further contribute to the understanding of the mechanisms of action of cover crops and mulching. In addition, the project aims to improve awareness and understanding among scientists and the general public (including farmers) of the concepts of organic agriculture, alternative agricultural practices, as well as the importance of microorganisms for proper soil processes, and the value that new molecular methods can offer. The successful implementation of the project will lay the foundation for innovative strategies to address societal challenges, including the reduction of adverse environmental impacts, adaptation to climate change, and the promotion of sustainable organic agriculture in Bulgaria.

Keywords: Organic farming; mulching; biodiversity; DNA barcoding and metabarcoding.

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OP–05

DOES CYTOSINE METHYLATION PLAY A ROLE IN REGULATING FLOWERING TIME UNDER WATER DEFICIT IN *ARABIDOPSIS THALIANA*

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This study addresses the role of cytosine methylation in the fine tuning of flowering time under water deficit in *Arabidopsis thaliana*. A *drm1 drm2 cmt3* triple methylation mutant was used together with Col-0 wild type. The plants were grown under long day conditions with water deficit induced by cessation of watering starting at 12 days after seeding. Col-0 showed 1 day delay in flowering as a result of the treatment. The *ddc*, on the other hand, showed 2 days of delay regardless of the experimental conditions. We found that the two b-box domain proteins BBX16/COL7 and BBX17/COL8 became overexpressed in the *ddc* background and in Col-0 under water deficit at 24 days after seeding. Additionally, the NF-YA2 transcription factor became correspondingly down-regulated. Our results support a model, where BBX16/COL7 and BBX17/COL8 interact with CONSTANS to delay the induction of *FT* under long day conditions. *NF-YA2*, which is also recognized as a promoter of *FT* expression, with its down-regulation causes additional delay of *FT* induced flowering. In the Col-0 background, the weak FRIGIDA allele fails to induce sufficient expression of *FLC* for additional suppression of *FT*. The plants overcome easily the BBX/NF-YA inhibition resulting in a relatively small delay in flowering. The expression patterns of the three genes suggest involvement of cytosine methylation in their regulation, however no differential methylation could be found in cis that can explain these effects. The results therefore, suggest a *trans* acting mechanism. Considering that the activities of *BBX16/COL7* and *BBX17/COL8* in different physiological conditions are not elucidated, this paper provides a background for future experiments targeting the role of these genes in the fine tuning of flowering time in *A. thaliana*.

Keywords: Abiotic stress; *Arabidopsis thaliana*; cytosine methylation; drought; epigenetics; flowering; water deficit

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OP–06

THE EXOCYST TETHERING COMPLEX AS A DETERMINANT OF BRASSINOSTEROID RECEPTOR TRAFFICKING IN PLANTS

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Brassinosteroids (BRs) are plant hormones with an impact on plant growth, development, and stress adaptation. These hormones bind to the plasma membrane-localized receptor kinase BRASSINOSTEROID INSENSITIVE1 (BRI1) which leads to formation of active BR signaling complexes. The complexes trigger a cascade of post-translational modifications which ensure transmission of the hormonal signal from the cell surface to the nucleus. It is known that the biologically active pool of BRI1 is located at the plasma membrane, while receptor internalization leads to reduced sensitivity to hormonal stimulus. Most of the BR receptor pool is subjected to continuous trafficking from the plasma membrane to intracellular compartments and back, and only a small fraction of BRI1 is targeted to the vacuole to ensure the protein turnover. Apart from the conventional endocytic, secretory and recycling pathways, autophagy also seems to be important for BRI1 desensitization, especially under stress conditions. In order to get novel insights into the versatile mechanisms of BRI1 trafficking regulation, we addressed possible functional interaction of the receptor with the exocyst, a tethering complex with a conserved role in intracellular vesicle transport across eukaryotes. To this end, we generated *Arabidopsis thaliana* marker lines with fluorescently tagged BRI1 in the context of impaired expression of individual exocyst subunits, such as SEC8, SEC15, EXO84, and EXO70. By using advanced live cell imaging techniques, we observed abnormal intracellular localization of BRI1 in the *exo70a1* and *exo84b* mutant lines, while the other studied exocyst mutants did not differ from the control genotype. Furthermore, we observed differential sensitivity to brassinolide treatment for two of the mutant lines with respect to their growth in the early stage of seedling development. Besides, pharmacological approaches revealed the substantial impact of the EXO70B1 subunit on BR receptor recycling. In addition, we studied the significance of EXO70B1 for the autophagic flux of BRI1 in conditions of salt stress or nitrogen deficiency. Our results suggested a major role of this exocyst subunit for BRI1 targeting to autophagosomes upon induction of autophagy. Overall, the obtained data demonstrate the importance of the exocyst complex for the intracellular trafficking of the brassinosteroid receptor in both optimal and adverse environmental conditions.

Keywords: Brassinosteroids; BRI1; Exocyst; Autophagy

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OP–07

MITOPHAGY IN RESURRECTION PLANT *HABERLEA RHODOPENSIS*: VACUOLAR INTRUSION OF MITOCHONDRIA RESPONDS TO CHILLING STRESS

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Haberlea rhodopensis is a homoiochlorophyllous resurrection plant with remarkable tolerance to both desiccation and sub-zero temperatures. While the overlap between drought and freezing tolerance has been previously established, specific mitochondrial responses to chilling stress remained poorly understood. This study elucidates the chilling tolerance mechanisms of *H. rhodopensis*, focusing on mitochondrial behaviour and alternative oxidase (AOX)-mediated respiration. Under chilling conditions (above-freezing low temperatures), *HrAOX2* transcript abundance, AOX protein levels and AOX-dependent respiration significantly increased—up to four-fold—compared to control plants. This shift in mitochondrial activity occurred alongside localized warming in the leaves, as detected by thermal imaging, suggesting that AOX activity contributes to cellular heat generation even in non-thermogenic plant tissues. Crucially, ultrastructural analysis revealed the unusual relocation of mitochondria into the central vacuole, where they appeared to undergo a slow and spatially controlled degradation process. This "delayed mitophagy" likely enables mitochondria to maintain AOX activity and contribute to thermogenesis and redox homeostasis before their eventual breakdown. The vacuolar sequestration of mitochondria may also protect the cytoplasm from excessive reactive oxygen species production during stress. Upon prolonged exposure or sub-zero temperatures, these mitochondria disintegrated, correlating with reduced AOX activity and loss of thermal buffering capacity.

Our findings reveal a sophisticated, multi-level adaptive strategy in *H. rhodopensis*, where coordinated AOX-driven respiration and regulated mitophagy support metabolic stability and thermal protection during chilling stress. This mechanism likely co-evolved with desiccation tolerance and may inform future approaches for improving cold resilience in crop species under climate change.

Keywords: *Haberlea rhodopensis*; mitophagy; alternative oxidase; thermogenesis, chilling stress

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OP–08

MICROALGAE IN SUSTAINABLE AGRICULTURE

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In the last decades, following the results of intensive agriculture, demand for sustainable alternatives to synthetic agrochemicals has grown. Microalgae as an indispensable part of soil and water and as producers of a wide range of biologically active substances take their place as potential biostimulants in the study of the interaction between microorganisms and plants. In this study microalgae from Laboratory of Experimental and Applied Algology collection were evaluated for their biostimulant potential. The aim was to investigate the effect of the red microalga *Porphyridium aerugineum* and green microalga *Chlorella vulgaris* fractions on the germination and early growth of *Lactuca sativa* seeds. Cell culture, disintegrated cell culture, biomass, disintegrated biomass, and supernatant of both microalgae were applied at three concentrations (0.5, 1, and 2 g/L). Results revealed that treatments with biomass, disintegrated biomass and supernatant significantly enhance seed germination and vigor. Specific effects were also observed – biomass of *Chlorella vulgaris* showed significant effect on *Lactuca sativa* dry biomass while *Porphyridium aerugineum* showed a higher positive effect on roots development. Biochemical analysis of different metabolite groups showed elevated levels of exopolysaccharides, lipids and phytohormones as auxins, gibberellins, cytokinins, and jasmonic acid. These findings confirm the distinct metabolic contributions of each species and demonstrate their potential as biofertilizers and agricultural biostimulants sources.

Keywords: Microalgae; biostimulant; *Chlorella vulgaris*; *Porphyridium aerugineum*; phytohormones; *Lactuca sativa*.

OP–09

PHYSIOLOGICAL AND BIOCHEMICAL RESPONSES OF RAPESEED PLANT UNDER HERBICIDE STRESS ARE MODULATED BY PRE-APPLICATION OF MICROBIAL BIOSTIMULANT

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The use of herbicides in agriculture significantly improves weed control; however, these chemicals can also negatively impact plant physiology. Therefore, this study aimed to analyze the effects of a herbicide, containing metazachlor (500 g/l), aminopyralid (5.3 g/l), and picloram (13.3 g/l), on various physiological and biochemical parameters in rapeseed plants. Additionally, the study examined the potential of the environmentally friendly microbial biostimulant "ProbioHumus" to mitigate herbicide-induced stress. The biometrical parameters, ethylene content, photosynthetic pigments (chlorophyll *a*, chlorophyll *b*, carotenoids content, a/b ratio), oxidative stress markers (malondialdehyde – MDA and hydrogen peroxide), osmolyte accumulation (proline), and components of the non-enzymatic antioxidant defense system (total phenolic compounds, antioxidant capacity) were assessed. Herbicide treatment had a negative impact on aboveground height, fresh and dry weights, which was mitigated by pre-application of microbial biostimulant. Results showed that ethylene levels doubled after herbicide treatment, indicating its role as a stress response even at the manufacturer's recommended dosage. Herbicide treatment combined with the microbial biostimulant reduced ethylene levels by 12% compared to controls. Herbicide treatment caused a statistically significant decline in chlorophylls and carotenoids content, whereas the microbial biostimulant moderated these negative effects. Markedly, oxidative stress markers increased substantially—MDA by 2.4-fold and hydrogen peroxide by 6-fold—following herbicide application, while combined treatment resulted in only a 27% increase of MDA, with hydrogen peroxide levels remaining almost at the same level as in the control plants. Proline content also increased under herbicide stress, but remained lower in plants treated with both herbicide and "ProbioHumus". The analysis of total phenolic content and antioxidant capacity suggests that the non-enzymatic antioxidant defense system responses to both herbicide and microbial biostimulant treatment. These findings demonstrate that the microbial biostimulant "ProbioHumus" can alleviate herbicide-induced damage, stabilize physiological functions, and promote stress tolerance in plants. This supports its potential role in sustainable agricultural practices and the gradual reduction of chemical herbicide usage.

Keywords: Herbicide; Microbial biostimulant; Oxidative stress; Rapeseed plant; Sustainable agriculture.

Acknowledgements: This work was supported by the Bulgaria-Lithuania joint research project IC-LT/02/2025-2027.

OP–10

ANTIFUNGAL POTENTIAL OF *NELTUMA HASSLERI* AGAINST CLINICALLY RELEVANT OPPORTUNISTIC FUNGI

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The global rise in opportunistic fungal infections poses a growing public health concern due to their high morbidity and mortality, especially in immunocompromised individuals. These infections, which range from superficial to life-threatening systemic diseases, are increasingly difficult to treat because of the emergence of drug-resistant strains. A major obstacle is the ability of fungi to form biofilms on both biotic and abiotic surfaces, enhancing resistance, persistence, and infection recurrence. Thus, the search for novel antifungal and antibiofilm agents is crucial. Medicinal plants represent a promising source of new compounds to improve therapeutic efficacy and combat resistance. This study aimed to evaluate the antifungal activity of the methanolic (MeOH) leaf extract of *Neltuma hassleri* (Fabaceae) which was collected and taxonomically identified in Santa Fe Province, Argentina, against *Candida* spp. and *Cryptococcus neoformans*. We also aimed to detect active compounds through bioautography, purify them by bio-guided extraction, and assess their biofilm-inhibitory potential.

Antifungal activity of the MeOH extract was evaluated using the broth microdilution method, showing minimum inhibitory and fungicidal concentration (MIC/MFC) values of 250/500 $\mu g.ml^{-1}$ against *Candida albicans* and 7.8/15.6 $\mu g.ml^{-1}$ against *Cryptococcus neoformans*, respectively. Thin-layer chromatography revealed the presence of active alkaloids, which were confirmed by bioautography and the Dragendorff reagent. From the MeOH extract, fractions enriched in alkaloids were prepared, obtaining MIC/MFC values as low as 15.6/31.2 $\mu g.ml^{-1}$ against *Candida albicans* and 0.97/0.97 $\mu g.ml^{-1}$ against *Cryptococcus neoformans*. Biofilm inhibition and eradication were evaluated in 96-well polystyrene plates using a biofilm-forming strain of *Candida parapsilosis* and the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The MeOH extract and alkaloid-enriched fractions inhibited biofilm formation and disrupted preformed biofilms in a dose-dependent manner. These findings highlight the antifungal potential of *N. hassleri* and foster further studies for isolation and characterization of its bioactive compounds.

Keywords: *Neltuma hassleri*; Alkaloids; Antifungal; *Candida albicans*; *Cryptococcus neoformans*.

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OP–11

ASSESSMENT OF DOSE-DEPENDENT INFLUENCE OF SPERMINE ON THE GERMINATION OF WHEAT SEEDS AND THE MYCELIAL DEVELOPMENT OF PATHOGENIC *FUSARIUM* FUNGUS

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Wheat (*Triticum aestivum* L.) being an essential global staple crop has been intensively cultivated. However, monoculture and climate change increases its vulnerability to biotic stressors, particularly diseases induced by pathogenic *Fusarium* spp., which jeopardizes production and grain quality through root and base rot. Conventional chemical fungicides encounter resistance challenges and environmental constraints, yet biological options such as seed priming using natural metabolites are receiving increased popularity. Polyamines, such as putrescine, spermidine, and spermine, are potential priming agents that may benefit plant growth and responses to both abiotic and biotic stress. Recently, exogenous addition of spermine is shown to possess antifungal activity against Fusarium, in vitro. This research investigated the efficacy of the polyamine spermine as a seed treatment to improve wheat resilience, with the objective of identifying a sustainable alternative to synthetic fungicides. Our main aim was to optimize spermine concentration for seed priming, since it is essential to enhance protection against *Fusarium*, while in addition promoting vigorous plant growth. We used a wide range of 0.5–5 mM spermine solutions and established that a six-hour seed soak in 5 mM dose markedly enhanced root weight and length of winter wheat seedlings. Moreover, we assessed the antifungal efficacy of spermine against three *Fusarium* species using *in vitro* tests. The findings demonstrated total suppression of *Fusarium culmorum* proliferation at 5 mM spermine. A minimal effect on *F. graminearum* and negligible influence on *F. oxysporum* was observed. Finally, we observed that the effect of the polyamine is fungistatic, meaning it limits the proliferation of mycelial growth of *F. culmorum* both *in vitro* and *in vivo*. This approach proved the effectiveness of spermine seed priming as an innovative tool for controlling Fusarium infections in wheat.

Keywords: *Fusarium* spp.; mycelial growth; seed priming; spermine; wheat.

Acknowledgements: This research was supported by the Bulgarian National Science Fund under contract KP-06-N86/6, dated 06 December, 2024.

POSTER PRESENTATIONS

PP–01

ORGANIC NANOPARTICLE-ASSISTED STRESS MODULATION IN *STEVIA REBAUDIANA*: BIOCHEMICAL IMPACTS OF M6 AND M6+IAA IN *IN VITRO* CULTURES

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This study explores the biochemical and physiological responses of *Stevia rebaudiana* plantlets to treatment with organic nanofibers M6 and its auxin-conjugated form, M6+IAA, during *in vitro* cultivation. Organic nanofibers, denoted as M6, composed of self-assembled peptidomimetics containing L-valine and nicotinic acid residues, were employed as carriers of the auxin IAA to support *in vitro* propagation of *Stevia rebaudiana*. The research aims to evaluate the potential of these nanoparticles in enhancing stress tolerance and promoting biochemical stability in plant tissues, which is particularly relevant for optimizing micropropagation protocols and increasing the overall efficiency of plant tissue culture. Stem explants were cultured on Murashige and Skoog (MS) medium supplemented with three concentrations (0, 1, 10, and 50 mg L^{-1}) of M6 or M6+IAA. Key physiological stress markers, including hydrogen peroxide (H_2O_2), malondialdehyde (MDA), proline, and sulfhydryl (SH-) compounds, were quantified using spectrophotometric methods based on established protocols.

The findings revealed that treatment with both M6 and M6+IAA led to a notable reduction in H_2O_2 and MDA levels, particularly at higher concentrations, indicating a decrease in oxidative stress. This antioxidant effect is likely linked to the molecular structure of M6, which includes nicotinic acid derivatives with known free-radical scavenging properties. In parallel, M6 and M6+IAA treatments modulated proline accumulation—a critical osmoprotectant—in a concentration-dependent manner. Specifically, proline levels increased under 10 mg L^{-1} M6 and 1–10 mg L^{-1} M6+IAA treatments, suggesting enhanced osmotic adjustment. However, a decline in SH-group content was observed across all nanoparticle treatments, potentially indicating redox shifts or sulfur metabolism changes.

Overall, the results support the use of M6-based nanoparticles, especially when combined with auxins, as promising agents for mitigating *in vitro* stress and improving the physiological quality of *Stevia rebaudiana* plantlets. These findings contribute to a growing body of evidence on the utility of nanotechnology in plant tissue culture, with possible applications in improving clonal propagation efficiency, stress resilience, and secondary metabolite production in economically important crops.

Keywords: Nanoparticles; *in vitro* stress tolerance; auxin conjugates; proline accumulation.

Acknowledgements: This work was conducted with financial support from National Science Fund at the Bulgarian Ministry of Education and Science, Project КП-06-Н56/8 12.11.21

PP–02

SPERMINE SEED PRIMING MITIGATES *FUSARIUM CULMORUM* STRESS IN WHEAT

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Fusariosis, caused by *Fusarium species*, is a major disease impacting cereal crops. While chemical fungicides are commonly used for seed treatment, an emerging, environmentally friendly approach is seed priming with substances, such as polyamines. This study investigated the efficacy of 5 mM spermine in priming wheat seeds to modulate stress responses against *Fusarium culmorum* infection. The pathogen was introduced by seed surface inoculation. *Fusarium culmorum* significantly increased stress markers (malondialdehyde, free proline, and electrolyte leakage) in the leaves of one-month-old plants. Additionally, *F. culmorum* infection substantially decreased plant fresh weight and height. These biometric changes coincided with a significant increase in the enzymatic activities of peroxidase (POX) and superoxide dismutase (SOD). Catalase (CAT) activity showed diverse responses, with a decrease in seed-infected plants. Seed priming with spermine is capable of alleviating these stress-induced alterations, bringing the assessed parameters closer to control levels and beneficially regulating the antioxidant defense system. These findings suggest that spermine seed priming is a promising approach to reduce disease severity caused by *Fusarium culmorum* in wheat.

Keywords: Antioxidant enzymes; *Fusarium culmorum*; seed pre-treatment; spermine; stress markers; wheat

Acknowledgements: This research was supported by the Bulgarian National Science Fund under contract KP-06-H86/6, dated 06 December, 2024.

PP–03

RESPONSE OF EINKORN AND WINTER WHEAT PLANTS TO TREATMENT WITH PLANT GROWTH REGULATOR MEIA AND NH_4NO_3 FERTILIZATION

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During the 2023-2024 growing season, field experiments with winter wheat (*Triticum aestivum* L., cv. Enola) and einkorn (*Triticum monococcum* L.) crops were conducted. The experimental area for each crop was 20 m², which was distributed in four variants of 5 m² each. The aim of the study was to investigate plant responses to treatment with the plant growth regulator MEIA (β-monomethyl ester of itaconic acid) and the application of ammonium nitrate using morpho-physiological and phytopathological approaches. The experimental scheme consisted of the following variants: 1. Control (untreated plants); 2. Plants that received NH_4NO_3 fertilizer during the tillering phase and were not treated with MEIA; 3. Plants that did not receive NH_4NO_3 fertilizer and were treated with MEIA twice during the stem elongation and grain filling phases; 4. Plants that received NH_4NO_3 fertilizer and were treated with MEIA during the stem elongation and grain filling phases. Fertiliser + MEIA treatments resulted in higher values of chlorophyll index, fresh and dry leaf mass, and leaf area. A strong positive effect of the applied plant growth regulator MEIA was observed in winter wheat, but the effect found in einkorn was weaker. The highest grain weight per plant was recorded under combined fertilization and treatment with MEIA in wheat Enola, while in einkorn, the most positive effect on grain weight per plant was achieved after treatment with MEIA. The combined effect of fertilization and MEIA on the activity of nitrate reductase and glutamine synthetase, two key enzymes in nitrogen assimilation, was also studied. The results showed that wheat was more responsive to N-fertilization and MEIA treatment compared to einkorn regarding both enzymes. Additionally, the causative agent *Blumeria graminis* f. sp. *tritici* (naturally occurred infection) significantly damaged the leaves in the control wheat plants (40.0 - 50.0% infected leaf area), while in all einkorn variants as well as in wheat treated with MEIA no damages were found. Further, the vitality of seeds obtained from plants subjected to the abovementioned treatments was estimated by germination tests during the sowing period and after storing at -20°C for six months. It was found that wheat seeds treated with MEIA germinated better and developed more robust coleoptiles (with greater fresh and dry weight) than those in the control group, while in einkorn only seed germination rate was higher. Also, treatment with MEIA increased the content of total phenols and anthocyanins in einkorn, but in wheat these effects were less noticeable and were expressed only in anthocyanin content.

Keywords: Einkorn; winter wheat; MEIA; germination; *Blumeria graminis* f. sp. *tritici.*

Acknowledgements: This research was supported by Bulgarian National Science Fund (BNSF), Grant No KP-06-N56/15

PP–04

THE EFFECT OF POLYAMINE SPERMINE ON KEY PHOTOSYNTHETIC PARAMETERS IN *FUSARIUM* INFECTED WINTER WHEAT

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Photosynthesis is a fundamental physiological process in plants, which can easily be affected by various environmental factors including biotic stressors. The application of plant growth regulators can benefit photosynthetic performance under both optimal and stress conditions. It is well known that the polyamine spermine positively influences photosynthesis. We evaluated the effect of seed priming with 5 mM spermine on photosynthesis-related parameters in wheat plants grown from seeds infected with the pathogenic fungus *Fusarium culmorim*. Under non-stress conditions, spermine priming improved leaf gas exchange, chlorophyll a fluorescence and pigment content compared to controls. Conversely, unprimed seedlings exposed to *F. culmorum* showed reduced pigment content, net photosynthesis rate, transpiration rate, stomatal conductance, as well as overall photosynthetic efficiency. Spermine-primed seeds exposed to *F. culmorum* displayed alleviation of the adverse effects on all measured parameters. These results validate the effectiveness of spermine seed priming as an effective strategy to modulate photosynthetic processes and mitigate biotic stress provoked by *F. culmorum* in wheat.

Keywords: *Fusarium culmorum*; photosynthesis; seed pre-treatment; spermine; *Triticum aestivum* L.

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PP–05

DROUGHT STRESS EFFECTS ON ENZYMATIC ANTIOXIDANTS IN BULGARIAN WINTER WHEAT (CV. FERMER) ARE MODULATED BY MELATONIN ROOT PRE-TREATMENT

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Climate change has intensified global water scarcity, profoundly affecting plant physiology and productivity. This is particularly critical for wheat, a major staple crop. To alleviate the adverse effects, plants activate defense mechanisms, encompassing enzymatic and non-enzymatic antioxidants. Melatonin, a well-known antioxidant, plays a role in improving plant tolerance in water-scarce environments. In this study, roots of young plants of the drought-sensitive Bulgarian winter wheat cultivar Fermer were treated with 75 µM melatonin and exposed to five days of water deprivation followed by a four-day recovery period. We analyzed the activities of catalase (CAT), guaiacol peroxidase (POD) and superoxide dismutase (SOD), as well as the expression of genes encoding antioxidant enzymes: *SOD-Cu-Zn*, *SOD-Mn*, *SOD-Fe*; *POD1*, *GPX*; *CAT3*, *CATA* in the leaves. Drought stress mostly elevated the activities of SOD and POD, with a modest rise in CAT, whereas during recovery, these activities returned to near-control values. Under drought conditions, gene expression was upregulated (*CAT3* and *CATA*), downregulated (*POD1* and *GPX*) or unaffected (*SOD* genes), whereas after recovery, expression levels approached normalization. Melatonin treatment did not significantly affect gene expression during drought but sustained elevated transcript levels of the relevant antioxidant enzymes post-recovery, suggesting a role in supporting recovery processes.

Keywords: *Triticum aestivum*; drought; melatonin; enzymatic antioxidants; gene expression.

Acknowledgements: This work was supported by the Bulgarian National Science Fund (Grant KP-06-N66/7).

PP–06

BLUE-RED LED LIGHT TRIGGERS PHYSIOLOGICAL, PHYTOCHEMICAL AND STRUCTURAL ADAPTIVE RESPONSES IN THE MEDICINAL PLANT *NEPETA NUDA*

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Light quality and duration profoundly influence the growth and productivity of plant species. This study investigated the effects of a blue-red LED light combination, known to induce flowering, on the physiological state and content of biologically active sub-stances in catmint (*Nepeta nuda* L.) grown under controlled in vitro conditions. White light (W) as a control was compared with two blue-red intensities: BR (blue-red light with hight intensity) and BRS (blue-red light with low intensity or "BR with shadow"). BR-treated plants showed increased leaf area and mesophyll thickness, higher biomass and starch content, but reduced levels of plastid pigments. BR also modified the oxidative state of plants by inducing lipid peroxidation while simultaneously activating ROS scavenging mechanisms and enhancing phenolic antioxidants. Interestingly, BR decreased the accumulation of the *Nepeta* sp. specific iridoid, nepetalactone. These effects appear to be regulated by the phytohormones auxin, abscisic acid and jasmonates. BRS treatment produced effects similar to the W control, but led to increased plant height and reduced leaf area and thickness. Both BR and BRS treatments regimes induced the accumulation of proteins and amino acids. We conclude that BR blue-red light can enhance the survival capacity of micropropagated *N. nuda* during subsequent soil adaptation, suggesting that similar light pre-treatment could improve plant performance under stress conditions.

Keywords: Leaf anatomy; phenolic compounds; phytohormones; plastid pigments; ROS; volatiles.

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PP–07

PROTECTIVE MECHANISMS IN ROOTS CONTRIBUTING TO THE RECOVERY OF *HABERLEA RHODOPENSIS* FROM DESICCATION

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The protective mechanisms in roots during rehydration of dried *H. rhodopensis* plants were investigated with the aim of better understanding the adaptive strategies of resurrection plants to survive desiccation. Ultrastructure analysis showed that the recovery of cortical cells is slower than in the central cylinder and transport tissue cells. Upon rewatering of roots, electrolyte leakage increased immediately and reached the control level in 1 h, indicating that the cell membranes were very well restored. The soluble carbohydrate content in dry roots was almost twofold higher compared to that in completely rehydrated plants. It increased further after 1 h of rehydration and remained high for up to 5 h, suggesting their importance in preserving proteins and membranes during root rehydration. In addition, a high amount of anthocyanins, flavonoids and ascorbate was determined during the recovery of roots. Moreover, the activity of superoxide dismutase and glutathione-S-transferase increased during the first 3 h of rehydration. Regardless of some variability, the total activity of glutathione reductase remained high during the rehydration of desiccated roots. It should be noted that even when the total activity of antioxidant enzymes was unaffected or lower compared to the control, the activity of specific isoenzymes was upregulated, which could be more beneficial under stress due to their action in specific cellular compartments. Western blot was used to identify the changes in the protein pattern of dehydrins, a LEA2 group proteins, during rehydration of *H. rhodopensis* roots. Several bands in the range of 17-60 kDa were detected. The most pronounced bands in all of the samples were the low molecular weight dehydrins with a mass around 18-25 kDa. Rehydration did not significantly change the protein profile of expressed dehydrins, but after 7 h of recovery, the intensity of all of the bands started to decline slightly. The most prominent increase in the amount of ELIPs was observed after 3 h of rehydration, when new ELIP isoforms appeared. The abundance of two sHSP proteins, molecular chaperones, was monitored in the course of rehydration of *H. rhodopensis* roots. The results showed that the content of the sHsp 17.7 Class II protein was enhanced after 3 h of rehydration and remained higher up to 7 h, while the content of sHSP 17.6 Class I protein strongly increased after 5 h of rehydration. In conclusion, enhanced antioxidant defense and accumulation of stress-induced proteins during the first hours of root rehydration contribute to their fast recovery.

Keywords: Drought stress; rehydration; root anatomy; non-enzymatic antioxidants; antioxidant enzymes; protective proteins.

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PP–08

SEED PRE-TREATMENT WITH SPERMINE MODULATES STRESS OUTCOME IN *TRITICUM AESTIVUM* L. PLANTS INFECTED WITH *FUSARIUM CULMORUM*

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Infection with pathogenic fungi from the genus Fusarium spp. causes fusariosis, one of the major diseases affecting cereal crops. Seeds are commonly treated with chemical fungicides to prevent disease. Priming with environmentally friendly compounds is an emerging approach aimed at enhancing plant growth, development and productivity under adverse conditions. A novel strategy for seed priming involves the use of polyamines, such as spermine. We applied 5 mM spermine to prime wheat seeds to modulate stress outcomes due to infection caused by *Fusarium culmorum*. The pathogen was applied through either seed surface inoculation or soil contamination. Regardless of the application method, *F. culmorum* significantly increased the content of stress markers (malondialdehyde, free proline, and leakage of electrolytes) and non-enzymatic antioxidants (total phenolics and free thiol-containing groups) in the last fully developed leaves of eight-week-old infected plants. The negative effects were more pronounced in plants grown from inoculated seeds. Seed priming with spermine partially alleviated the stress-induced increase of the assessed parameters. It could be concluded that priming with spermine is a promising approach to mitigate the effects of the infection caused by *F. culmorum*.

Keywords: *Fusarium culmorum*; seed pre-treatment; spermine; stress markers, *Triticum aestivum* L.

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PP–09

PROFILING OF ANTIOXIDANT ENZYMES-CODING GENES IN DROUGHT-STRESSED *ARABIDOPSIS THALIANA* PLANTS WITH CELL TYPE-SPECIFIC ATTENUATION OF ETHYLENE SIGNALING

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Plant survival under stress depends on the efficient adjustment of physiological processes to align with changing environmental conditions. These responses are orchestrated by a complex network of hormonal and metabolic signals. During drought, plant stress responses focus primarily on mitigating osmotic and ionic imbalances and managing oxidative stress at the cellular level. The role of ethylene signaling in triggering adaptive physiological responses to dehydration, including the activation of the antioxidant defense system, remains insufficiently explored.

We investigated drought-induced changes in transcripts encoding antioxidant enzymes in transgenic *Arabidopsis thaliana* lines with cell-type-specific attenuation of ethylene signaling. Transcript levels of genes coding for a superoxide dismutase (*SOD1*, AT1G08830), peroxidase (*PER57*, AT5G17820), and catalase (*CAT1*, AT1G20630) were measured in the leaves of control and drought-stressed plants using RT-qPCR. The analyzed transgenic lines have reduced ethylene sensitivity in specific root cell types: epidermis (*pA14:EBF2*, *pLRC1:EBF2*), quiescent center (*pQ6:EBF2*), root auxin maximum (*pDR5:EBF2*), cortex (*pCOR:EBF2*), endodermis (*pE30:EBF2*), pericycle (*pS1:EBF2*) and vasculature (*pS2:EBF2*) (Vaseva et al., 2018). Growth parameters (leaf area, fresh and dry weight) and stress markers (MDA, L-proline, H_2O_2) in the leaves of wild-type plants (Col-0), the ethylene-insensitive double mutant *ein3eil1*, and the transgenic lines were evaluated under moderate soil drought (30% field capacity) imposed for seven days.

Under water-limiting conditions, lines with reduced ethylene sensitivity in the epidermis (*pA14:EBF2*, *pLRC1:EBF2*) and pericycle (*pS1:EBF2*) were the least affected by dehydration. Transcript profiling of antioxidant genes indicated that the better performing transgenic lines maintained stable or slightly reduced *CAT1* and *PER57* transcript levels under dehydration, in contrast to the observed up-regulation of these genes in the wild type drought-stressed plants. The plants with impaired ethylene signaling in the cortex (*pCOR:EBF2*) showed the most pronounced growth reduction. The same transgenic line (*pCOR:EBF2*) had increased abundance of *SOD1* transcripts under stress. The experimental approach based on the use of these transgenic lines could outline other molecular players in ROS detoxification that operate in particular cell types and are dependent on ethylene signals therein.

Keywords: *Arabidopsis thaliana;* drought; ethylene signaling; genes for antioxidant enzymes; stress markers.

Acknowledgements: This research was funded by the Bulgarian National Science Fund (BNSF); grant number KP-06-N71/12/10 July 2024.

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PP–10

ROOT APPLICATION OF MELATONIN MODULATES NON-ENZYMATIC ANTIOXIDANTS AND GLUTATHIONE REDUCTASE IN WHEAT SUBJECTED TO DROUGHT STRESS

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The scarcity of water, as a crucial environmental factor, severely limits agricultural output. Drought stress adversely affects several aspects of plant biochemistry, which notably compromises antioxidant defense systems. Although wheat can withstand some level of water deficiency, prolonged droughts cause several detrimental changes in plants, resulting in a substantial decrease in yield. The search for ecologically friendly substances that might support normal physiological functions and alleviate adverse effects in plants under water shortage is of significant interest. Melatonin is a naturally occurring antioxidant that regulates plant growth and development. It takes part in the regulation of several physiological processes and it's function have lately been extensively studied. Young wheat (*Triticum aestivum* L.) cultivars Fermer and Gines were root-supplied with melatonin, and on the next day, the plants were subjected to a 5-day drought stress, followed by a 4-day recovery phase. The amount of total phenolics and free thiol-group-containing compounds, together with the activity and gene expression of glutathione reductase, were assessed immediately after drought and following recovery. Drought markedly elevated all assessed parameters in both cultivars, with a more noticeable effect in the drought-susceptible cultivar Fermer. The administration of melatonin mitigated the effects of drought to some extent. Subsequently, during the recovery period, the metrics generally approached control values, except that the glutathione reductase transcript levels were elevated in melatonin pre-treated plants of both cultivars.

Keywords: *Triticum aestivum*; drought; melatonin; non-enzymatic antioxidants; glutathione reductase.

Acknowledgements: This work was supported by the Bulgarian National Science Fund (Grant KP-06-N66/7).

PP–11

THE EFFECT OF SEED PRIMING WITH SPERMINE ON PHENOTYPICAL ALTERATIONS AND ENZYMATIC ANTIOXIDANT DEFENSE IN FUSARIUM-INFECTED WHEAT PLANTS

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Fusariosis caused by *Fusarium* spp. is one of the main constraints in cereal production. To prevent disease, seeds are routinely treated with chemical fungicides. Priming with ecologically safe substances is a relatively new technique for improving plant growth, development, and productivity under unfavorable growth conditions. A new approach for seed priming is the usage of spermine. We used 5 mM spermine to prime wheat seeds, aiming to boost antioxidant defense to cope with the following infection of *Fusarium culmorum*. The pathogen was inoculated directly on wheat seed or introduced into soil substrate. *Fusarium culmorum*, either seed- or soil-applied, substantially decreased the fresh weight and plant height of infected eight-week-old plants, with more severe effects caused by seed inoculation. The alterations in biometric parameters were in parallel with a significant increase in the content of hydrogen peroxide and the enzymatic activities of peroxidase and superoxide dismutase, which were more pronounced in the leaves of seed-contaminated wheat. Both types of pathogen infection caused diverse effects on catalase activity – a strong decrease in seed-infected plants, while a negligible increase was observed after soil contamination as compared to the control. Seed priming with spermine beneficially regulated the assessed parameters, which neared the control levels. In conclusion, priming with spermine might have the potential to reduce *Fusarium culmorum* disease in wheat.

Keywords: Antioxidant enzymatic activity; *Fusarium culmorum*; seed pre-treatment; spermine; wheat.

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PP–12

PRE-TREATMENT WITH MELATONIN MODULATES ENZYMATIC ANTIOXIDANTS IN DROUGHT TREATED WINTER WHEAT CV. GINES

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Drought stress is a primary environmental constrain that limits agricultural productivity. It has become a substantial issue in the wheat agricultural industry, given the essential role of this crop as a global food staple that feeds billions. Water scarcity leads to the accumulation of reactive oxygen species (ROS), which disrupt many facets of plant biochemistry. The production of antioxidant compounds and the activation of specialized enzymes protect plants from oxidative damage by neutralizing excess ROS and maintaining cellular homeostasis. The primary constituents of the enzymatic antioxidant defense system are the superoxide dismutase (SOD) family, catalase (CAT), enzymes of the glutathione-ascorbate cycle and various peroxidases (POX). The antioxidant system can be stimulated by bioactive molecules like melatonin. This study investigates how melatonin pre-treatment of roots affects enzymatic antioxidant activities and the expression of associated genes in young winter wheat plants (cv. Gines). Plants were exposed to drought for five days and then rehydrated for four days. Drought stress increased the activity of CAT, POX and SOD, as well as the expression of *CAT3*, *CATA* and *SOD-Fe*. Conversely, the expression of *POD1*, *GPX*, *SOD-Cu-Zn* and *SOD-Mn* was downregulated under these conditions. Following the recovery phase, enzymatic activities approached the control values; however gene expression levels remained elevated, except for *SOD-Cu-Zn*, which returned to baseline. Under drought conditions, melatonin pre-treatment reduced the activities of antioxidant enzymes compared to drought-stressed plants without melatonin. After rehydration, melatonin-treated plants maintained enzyme activities and gene expression levels around control levels, with the exception of *GPX*, *POD1*, *SOD-Mn* and *SOD-Fe* coding genes, which remained upregulated.

Keywords: *Triticum aestivum*; drought; melatonin; enzymatic antioxidants; antioxidant gene expression.

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PP–13

DIFFERENT RESPONSES TO DROUGHT IN TOLERANT AND SENSITIVE WHEAT VARIETIES: A COMPARATIVE ANALYSIS

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Climate change has led to an increase in the frequency and severity of drought posing a serious threat to agricultural productivity, especially in cereal crops like wheat. In this study, we investigated the physiological and biochemical responses of two wheat cultivars, *Katya* (drought-tolerant) and *Zora* (drought-sensitive) under controlled drought stress conditions. A series of biometric measurements were conducted, including fresh weight, dry weight, and leaf area. The results showed that *Katya* exhibited significantly better growth performance under drought conditions compared to *Zora*.

A detailed chlorophyll fluorescence analysis was performed to assess the impact of drought on the photosynthetic machinery. The following parameters were measured: F_o, Fm, Fv (Fm–F_o), Fv/F_o, Vj, Vi, $\phi(P_o)$=Fv/Fm, ψ_o=1–Vj, M_o, Ss, ABS/RC, TR_o/RC, ET_o/RC, DI_o/RC, ϕE_o, ϕD_o, PI(abs), and DF(abs). These parameters provided in-depth insights into the structural and functional changes in the photosynthetic apparatus during drought stress. Across most indicators, *Katya* demonstrated higher photosystem II stability, greater light energy conversion efficiency, and stronger overall stress resistance than *Zora*.

Thermoluminescence analysis further confirmed that *Zora* underwent significantly higher oxidative and photo-oxidative stress levels during drought. After several days of water deficit, both cultivars were rehydrated. Post-rehydration measurements revealed that *Katya* could restore its physiological and fluorescence parameters to near-baseline levels, indicating robust recovery capacity. In contrast, *Zora* showed minimal recovery and sustained damage, suggesting limited resilience.

Overall, the results highlight *Katya's* superior drought tolerance and ability to recover after stress, making it a promising candidate for cultivation in regions increasingly affected by climate change.

Keywords: Fluorescence; Thermoluminescence; Drought stress; Wheat.

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PP–14

SPERMINE PRIMING MODULATES PHOTOSYNTHESIS IN *TRITICUM AESTIVUM* L. GROWN ON SOIL INFECTED WITH *FUSARIUM CULMORUM* (Wm. G. Sm.) Sacc.

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Non-invasive biophysical methods are widely used to assess photosynthesis, a fundamental physiological process often influenced by diverse environmental challenges. The efficiency of photosynthesis under normal and stress environments can be increased by the application of various chemicals, for example plant growth regulators. The polyamine spermine is known to positively affect photosynthetic process. In this study, we evaluated the effect of seed priming with 5 mM spermine on leaf pigment content, net photosynthesis rate, transpiration, stomatal conductance and total photosynthetic efficiency in *Triticum aestivum* L. cv. Sadovo-1 grown in soil infected with *Fusarium culmorum*. Measurements were performed on the last fully developed leaf to assess photosystem functionality, photosynthetic efficiency and plant adaptation under biotic stress. In the absence of stress, spermine priming enhanced leaf gas exchange parameters, chlorophyll a fluorescence and pigment content relative to the control group. Conversely, plants grown in pathogen-infected soil without priming showed pronounced photosynthetic impairment. Seed pre-treatment with spermine mitigated these negative effects and improved photosynthetic performance under stress conditions. The combined use of physiological and biophysical methods confirmed the beneficial role of spermine seed priming as a modulator of photosynthesis in wheat plants under *F. culmorum*-induced stress.

Keywords: *Fusarium culmorum*; photosynthesis; seed priming; spermine; wheat.

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PP–15

GENOME-WIDE ANALYSIS OF GIBBERELLIN OXIDASE GENES AND HORMONAL RESPONSE TO GA_3 IN *HERACLEUM SOSNOWSKYI*

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Gibberellins (GAs) are vital phytohormones that regulate growth and development in plants. Their biosynthesis culminates in the formation of bioactive GA_4 and GA_1, which are tightly controlled by GA-oxidase enzymes – GA20ox, GA2ox, and GA3ox. In this study, we performed a genome-wide characterisation of these GAox enzymes, analysed their gene expression, and assessed endogenous GA levels in the highly invasive *Heracleum sosnowskyi* Manden. following exogenous GA_3 treatment.

We identified 27 GAox proteins in the *H. sosnowskyi* genome: nine GA20ox, thirteen GA2ox, and five GA3ox, all containing conserved 2OG-Fe(II) oxygenase (PF03171) and non-haem dioxygenase N-terminal (PF14226) domains. These genes are distributed across 11 chromosomes, with predicted localisation in the cytoplasm. Phylogenetic analysis grouped the proteins into three families – GA20ox, C_{19}-GA2ox, and GA3ox – while no C_{20}-GA2ox members were found. Gene structure and motif analyses revealed evolutionary conservation with distinct patterns among the families.

We successfully cloned *HsGA20ox1*, *HsGA3ox1*, and *HsGA2ox1*, which showed peak expression in developing ovaries. Expression analysis post-GA_3 treatment revealed dynamic regulation: HsGA3ox1 was strongly upregulated, whereas *HsGA20ox1* was downregulated 10 days after application. Endogenous GA profiling indicated increased GA_1 in lateral umbels and a decrease in central ones, while GA precursors (GA_{44}, GA_{19}, GA_{20}) and catabolites (GA_8, GA_{29}) accumulated in a stage and tissue-specific manner. Phenotypically, GA_3 treatment reduced ovary size and weight: by 21%, 30%, and 59% in central umbels and by 20%, 31%, and 41% in lateral ones, respectively.

This comprehensive analysis reveals how GAox genes mediate hormonal responses to exogenous GA_3 in *H. sosnowskyi*, impacting ovary development and potentially seed production. These insights advance our understanding of GA regulation in invasive species and support the development of targeted control strategies.

Keywords: Sosnowsky's hogweed; Invasive plant; Apiaceae; Plant hormone analysis; Gibberellic acid.

PP–16

EFFECT OF LIGHT SPECTRUM ON THE PHYSIOLOGICAL STATE AND BIOACTIVE POTENTIAL OF THE CARNIVOROUS PLANT *DROSERA ROTUNDIFOLIA*

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The genus *Drosera* consists of small carnivorous perennial plants growing in acidic, moist, and nutrient-poor environments, where they have evolved unique adaptations for survival, including the production of secondary metabolites with remarkable bioactivity. The light is a critical environmental factor regulating the growth and productivity of sundews, particularly in their naturally shaded to a different extent habitat. In this study, we investigated the effect of different light spectra on the morphology and total metabolite synthesis of *in vitro* cultivated *Drosera rotundifolia*. Plants were grown under white light, high-intensity blue-red and low-intensity blue-red lights. The pigment content, carbohydrate accumulation, and the synthesis of phenolic, flavonoid and anthocyanin compounds were monitored. Additionally, the antioxidant and antimicrobial potential of the plants’ extracts was evaluated using standard bioassays.

The results demonstrated that white light is optimal overall development of the plants, including pigment synthesis and biomass accumulation. In contrast, high-intensity blue-red light inhibited normal growth and metabolic activity was shifted towards anthocyanin biosynthesis. Under this light variant, fluorescent staining revealed elevated ROS accumulation in the leaf blade and reduced trichomes, which is likely related to stress response. Notably, exposure to low-intensity blue-red light stimulated the production of antimicrobial compounds, supporting a light-mediated enhancement of secondary metabolism under conditions that mimic the plant's native ones at low-light environment. These findings emphasize the pivotal role of light quality and intensity for modulating both primary and secondary metabolic processes in *Drosera rotundifolia*, offering potential for optimizing cultivation conditions for medicinal or biotechnological purposes.

Keywords: Antimicrobial activity; light; secondary metabolites; sundew.

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PP–17

EFFECT OF UV IRRADIATION (SINGLE AND COMBINED) ON MODEL AND WILD-GROWING PLANTS

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This study aimed to compare the effect of prolonged UV irradiation (single and combined), on photosynthetic pigment content, measured in a model plant under controlled laboratory conditions and wild-growing plants at high-mountain conditions. The objects of this study are one model plant, *Hordeum vulgare* L. (Poaceae), grown under standard laboratory conditions and ten wild-growing species (*Saxifraga cymosa* Waldst. & Kit. (Saxifragaceae), *Anthemis carpatica* Waldst. & Kit. ex Willd. (Asteraceae), *Geum reptans* L. (Rosaceae), *Doronicum columnae* Ten. (Asteraceae), *Achillea clusiana* Tausch. (Asteraceae), *Armeria alpina* Willd. (Plumbaginaceae), *Festuca valida* (R.Uechtr.) Pénzes (Poaceae), *Pedicularis orthantha* Griseb. (Scrophulariaceae) and *Campanula alpina* Jacq. (Campanulaceae)) at the Moussala Peak (2925 m a. s. l.) Rila Mountain. *H. vulgare* was used to assess the single effect of UV-irradiation at controlled laboratory conditions. After germination, barley seeds were grown in a growth chamber (12-h light/photoperiod/12-h dark; constant temperature; constant watering). 7-day-old plants were irradiated in a UV chamber for periods of 10, 20, 30 and 40 days. UV conditions simulated the solar UV irradiation at the Moussala Peak in the summer of 2023. Our previous observations of the effect of solar UV irradiation, measured as pigment content at this peak, showed that this content was higher in the summer of 2023 than in the summers of 2020, 2021, 2022, and 2024, in three of the ten wild plant species studied. Wild-growing plants were collected during five studied seasons (from July to August) of the years 2020-2024. The content of photosynthetic pigments (total chlorophylls, chlorophyll *a*, chlorophyll *b* and total carotenoids) was spectrophotometrically determined. The chlorophyll *a/b* ratio was calculated. Based on our results, it can be concluded that: 1. Sensitivity to UV irradiation varies depending on the studied conditions for the respective year, the duration of exposure and the genotype. A difference in the content of photosynthetic pigments was obtained between the model plant grown under single UV irradiation (controlled laboratory conditions) and wild plants growing in combined alpine conditions. 2. The wild-growing species show different tolerance to the combined effect of UV irradiation with other abiotic factors (alpine conditions), measured as levels of photosynthetic pigments. 3. UV irradiation increased the pigment content in a time-dependent manner under laboratory conditions. 4. Changes in the pigment content in wild-growing species at the Moussala Peak could be due to different adaptive mechanisms of the plants.

Keywords: UV irradiation; altitude; model plant; wild-growing plants; photosynthetic pigments.

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PP–18

BIOCROP: GENETIC AND METABOLOMIC INSIGHTS INTO SEAWEED BIOSTIMULANT PRIMING FOR STRESS TOLERANCE AND NUTRITIONALLY SUPERIOR CROPS

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Abiotic stresses, notably drought and heat, cause significant agricultural losses globally, severely impacting food security. Seaweed-derived biostimulants, such as SuperFifty (SF) from *Ascophyllum nodosum*, emerge as eco-friendly solutions promoting plant stress tolerance through molecular priming. Preliminary research demonstrated SF ability to significantly enhance stress tolerance (drought, heat, and oxidative stress) and improve marketable yield in major crops, including tomato, pepper, maize, raspberry, and strawberry. Functional characterization in *Arabidopsis thaliana* revealed key transcription factors (ERF54) specifically activated by SF-induced priming, coordinating protective responses against drought by repressing negative regulators like ubiquitin ligases (RGLG1/2). We propose an innovative, genome-wide approach combining mutant screening in *A. thaliana* and Genome-wide association analysis (GWAS) in diverse tomato and pepper germplasm (>300 lines) to identify the genetic determinants underlying SF-induced priming. Selected candidate genes from association studies will undergo validation through CRISPR/Cas9 and mutant analyses. Additionally, comprehensive transcriptomic and metabolite profiling (GC/UHPLC-MS, ICP-MS) will identify genes, pathways, and metabolites highly regulated during biostimulant-induced stress protection and crop enhancement. BIOCROPS aims to decode the genetic and metabolic networks driving biostimulant effectiveness, advancing sustainable agriculture by delivering stress-tolerant, nutritionally enriched crops.

Keywords: Biostimulant; Oxidative stress; GWAS; Metabolomics, Drought, Transcriptomics.

PP–19

MICROBIAL BIOSTIMULANT AND CALCIUM SYNERGY IN IMPROVING DROUGHT TOLERANCE OF WINTER CROPS

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The cultivation of economically important winter crops (wheat and oilseed rape) is increasingly challenged by extreme climatic conditions, as many productive varieties fail to fully express their genotype-determined potential (Papacz et al., 2008; Gavelienė et al., 2014). Climate-induced droughts and heat waves reduce plant growth and development, disrupt cereal ear and rapeseed pod formation, decrease seed mass, deteriorate oil quality, and lower nutritional value.

A novel approach to enhancing crop drought tolerance is the use of substances with adaptogenic effects in combination with calcium soil amendments. The aim of this study was to investigate the responses of winter oilseed rape (*Brassica napus* L., cv. 'Visby') and winter wheat (*Triticum aestivum* L., cv. 'Skagen') to prolonged simulated drought and to evaluate the effects of the microbial biostimulant ProbioHumus and calcium (Ca), applied individually and in combination.

Plants were grown under controlled conditions. Calcium carbonate ($CaCO_3$, 3.71 g pot^{-1}; 70 g m^{-2}) was applied to the soil, and at the 3–4 leaf stage seedlings were sprayed with ProbioHumus (2 ml 100 ml^{-1}). Drought was induced for 8 days, followed by re-irrigation and recovery assessment.

Results showed that ProbioHumus alleviated the physiological and biochemical effects of drought stress in oilseed rape. The combined ProbioHumus + Ca treatment increased leaf relative water content by 87%, enhanced proline accumulation (4-fold), improved photosynthetic pigment content (10–28%), reduced H_2O_2 (–53%) and malondialdehyde (–45%), and stimulated stomatal opening (2-fold upper, 1.4-fold lower epidermis), compared to drought control. This combination was the most effective in promoting survival and recovery after drought.

In winter wheat, all treatments improved drought tolerance, with the strongest effects observed in ProbioHumus and ProbioHumus + Ca variants. These maintained relative leaf water content and growth parameters at levels close to irrigated plants, delayed drought-induced ethylene emission, reduced ROS-related membrane damage, and downregulated drought-responsive genes.

In conclusion, the application of microbial biostimulants in combination with calcium activates defense mechanisms that mitigate the adverse effects of drought stress in winter crops, offering a promising strategy for climate-resilient agriculture.

Keywords: Adaptogenic effects; calcium soil amendments; drought stress; oilseed rape; plant probiotics; winter wheat.

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PP–20

EVALUATION OF THE EFFECT OF AQUEOUS EXTRACTS OF *ROSMARINUS OFFICINALIS* ON ROOT GROWTH OF *ARABIDOPSIS THALIANA* AND ITS POSSIBLE RELATIONSHIP WITH AUXIN SIGNALING

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In the current global context, there is a high demand for novel bioeffectors from natural sources. Extracts with organic solvents from *Rosmarinus officinalis* (Lamiaceae) have been extensively studied, but there is limited information on the biological activity of aqueous extracts from this species. Previous studies carried out by our research group have shown that foliar application of aqueous extracts of *Rosmarinus officinalis* induces immune responses in tobacco and other model plants. However, when these extracts are applied to roots, an inhibition of root growth is observed, with rosmarinic acid being the main active compound. The aim of the present study is to investigate the physiological and molecular mechanism responsible for this effect, using *Arabidopsis thaliana* as a model system. The aqueous extracts come from two different rosemary varieties (Criollo and Frances), originally found in different regions of Argentina, but cultivated under the same climatic conditions in the province of San Luis: R4 (Frances variety from Córdoba), R6 (Criollo variety from Salta), and ARE (Criollo variety from San Luis). In our experiments, rosmarinic acid was included as a positive control, given its well-known biological activity. Seedlings of *Arabidopsis thaliana* fluorescent marker lines associated with auxin signaling and transport were incubated in liquid MS medium with or without extracts for 2 and 24 hours to evaluate whether the biological activity of the extracts differs from that of pure rosmarinic acid at the same molarity. The analyses were performed in the root tip zone using confocal microscopy. In addition to evaluating auxin-related responses, this study aims to determine whether the biological activity of an aqueous extract of *Rosmarinus officinalis*, a complex mixture of compounds, significantly differs from that of the active compound rosmarinic acid at the same molarity.

Keywords: *Rosmarinus officinalis*; Aqueous extract; Rosmarinic acid; Auxins; Confocal microscopy.

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PP–21

IN VITRO CYTOSTATIC AND PROAPOPTOTIC PROPERTIES OF AQUEOUS EXTRACT FROM FLOWERS OF WILD-GROWN *NEPETA NUDA* L. TOWARDS CANCER CELL LINES

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Nepeta nuda L. (Lamiaceae) is a medicinal plant widely applied in traditional folk medicine due to its numerous therapeutic properties, including antioxidant, antimicrobial and antiviral activities, as well as to its wound healing effects. The anticancer potential of this valuable plant species has been not sufficiently studied (Gospodinova et al. 2024). The objective of the present work was to assess antiproliferative properties of wild-grown *N. nuda* aqueous flower extract on five cancer cell lines representing some of the most common and lethal cancer types: MDA-MB-231, MCF7 (breast cancer), HepG2 (hepatocellular carcinoma), Colon 26 and HT29 (colon adenocarcinoma) cell lines. The selectivity of the extract's cytostatic effect was evaluated on a non-cancerous skin cell line BJ after 72 h-treatment by MTT cell proliferation assay. Changes in the cell morphology were inspected by light microscopy. The obtained results indicate cytostatic effect of *N. nuda* flowers extract against studied cancer cell lines with the strongest cell growth inhibitory effect on Colon 26 cells. A significantly weaker reduction in the proliferation rate of non-cancerous cells BJ was observed following extract treatment. Notable alterations in Colon 26 cell morphology, along with a marked reduction in the monolayer cell density, were observed after 72 hours of treatment with the plant extract. In order to investigate the proapoptotic potential of *N. nuda* flowers extract, Colon 26 cancer cells were analyzed by flow cytometry after staining with Annexin V/propidium iodide. The results revealed significant induction of early apoptotic events in the cancer cells, following the extract application. A slight increase in the number of cells in late apoptosis state was registered as well. Further in-depth studies will be performed to elucidate the mechanisms of anticancer action and molecular targets of *N. nuda* flower extract.

Keywords: *Nepeta nuda* L.; cancer cell lines; antiproliferative activity; apoptosis.

Acknowledgements: This work was supported by the Bulgarian National Science Fund, Ministry of Education and Science, Grant No. KP-06-N56/9/12.11.2021.

References: Gospodinova Z, Antov G, Stoichev S, Zhiponova M (2024) *In vitro* anticancer effects of aqueous leaf extract from *Nepeta nuda* L. ssp. nuda. Life 14: 1539.

PP–22

ANTICANCER POTENTIAL OF *IN VITRO* CULTIVATED BULGARIAN MEDICINAL PLANTS

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Sideritis scardica Griseb. (Mursala tea), *Clinopodium vulgare* L. (Wild basil), and *Salvia aethiopis* L. (Mediterranean sage) are valuable Bulgarian medicinal plants with high pharmacological potential and broad-spectrum biological activity. These species are well-known for their antioxidant, antibacterial, neuroprotective, and anti-inflammatory properties. Despite their extensive use in traditional medicine and various biomedical studies, specific data about their potential in cancer prevention and treatment, especially regarding the cultivated plants, are not sufficiently investigated.

The anticancer potential of aqueous extracts obtained from *in vitro* propagated plants *C. vulgare*, *S. scardica*, and *S. aethiopis* was assessed on a panel of cell lines, including cervical adenocarcinoma (HeLa), colorectal adenocarcinoma (HT-29), breast carcinoma (MCF-7), and hepatocellular carcinoma (Hep-G2), as well as control non-cancerous cell lines.

The results demonstrated that the aqueous extracts from *in vitro* cultivated C. vulgare exhibit stronger antitumor activity compared to extracts from wild-grown plants. Significant inhibition of cancer cell viability, proliferation, and migration was observed, accompanied by cell cycle arrest and induction of apoptosis. Fluorescence microscopy and cell cycle analysis confirmed that the antitumor activity were associated with both antiproliferative and proapoptotic effects. In the case of *S. scardica*, extracts from *in vitro* cultivated plants displayed a pronounced, concentration-dependent cytotoxic effect on the HeLa cell line, while extracts from conventionally grown plants were most effective against MCF-7 breast cancer cells. In both cases, the non-tumor cell line was significantly less affected, indicating selectivity of the extracts toward cancer cells. The most pronounced activity against liver cancer cells was observed with aqueous extracts from cultivated *S. aethiopis*. These extracts selectively inhibited the proliferation of hepatocellular carcinoma cells through apoptosis, necrosis, and G2/M cell cycle arrest. Flow cytometry revealed increased populations of apoptotic and necrotic cells. Migration assays and cytopathological evaluations further confirmed significant suppression of cancer cell proliferation and migration, along with low toxicity toward normal cells.

The investigated *in vitro* cultivated Bulgarian medicinal plants demonstrate considerable antitumor potential, manifested through selective cytotoxicity, inhibition of cell migration, cell cycle arrest, and induction of apoptosis. These findings support the need for further research into the bioactive compounds of these species and their possible application in cancer therapy.

Keywords: Cultivated plants; anticancer activity; apoptosis; cell migration; cell cycle arrest.

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PP–23

PROMOTING BIODIVERSITY AND SUSTAINABILITY THROUGH VALORISATION OF ORPHAN LEGUMES (PROSPER) - BIG IDEAS FROM MODEST BEANS

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Ever heard of orphan legumes (OLs)? No? Do not worry – they are about to get the attention they deserve. The newly launched Horizon Europe project PROSPER, funded under the HORIZON-CL6-2024-BIODIV-02 call, aims to demonstrate the value of these tough, resilient and often overlooked crops. PROSPER will expand their use in agriculture and deliver a wide range of nutritious, sustainable and even biodegradable products, unlocking the full potential of OLs. The project spans Mediterranean, Central and Northern Europe, bringing together farmers, researchers and food innovators in a shared mission. PROSPER applies advanced technologies and practical expertise to address biodiversity loss and agricultural challenges, transforming European agriculture - legume by legume. Work Package 6 (WP6), led by the Institute of Plant Physiology and Genetics at the Bulgarian Academy of Sciences, focuses on product innovation. The team will investigate molecular gastronomy, fermentation and eco-friendly methods to create future foods, functional snacks, natural additives and biodegradable materials based on OLs. Sensory analysis and consumer feedback help refine these products to meet market demands and sustainability targets. By introducing OLs into new value chains and expanding their use, PROSPER, and particularly WP6, will strengthen the circular bioeconomy and support the development of a more diverse and resilient European food system.

Keywords: Orphan legumes; Functional foods; Sustainable agriculture; Sensory validation, WP6.

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PP–24

RED BLOOD CELLS AND MICROBIOME BIODIVERSITY

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The erythrocyte microbiome serves as a crucial indicator of blood health. Indirect evidence from radiometric studies further supports the presence of living microbial forms within erythrocytes. In our investigation, we analyzed both uncultured and cultured blood samples from clinically healthy individuals under stress conditions (43°C for 24 h). Our findings reveal that the microbiota circulating in the bloodstream possesses a well-defined cell wall and reproduces through budding or cell wall shedding mechanisms. When cultured under stress conditions, the blood microbiota proliferate as microbial cells lacking a cell wall, resulting in the formation of electron-dense or electron-bright bodies. These electron-dense bodies reproduce through fission, generating Gram-negative daughter cells, or by enlarging until they rupture, releasing daughter bodies. Additionally, we demonstrated that the blood microbiome encompasses a significant variety of bacterial species. We quantitatively measured the culturable part of blood microbiota of healthy individuals by culturing freshly drawn blood in Brain Heart Infusion medium supplemented with 10% sucrose and a high concentration of Vitamin K (1 mg/mL) and incubated at 43°C for 24 h. The explosive growth of microbial structures was observed by light microscopy on Gram-stained slides. To discriminate the culturable part of the blood microbiota we applied targeted sequencing of 16S rDNA and internal transcribed spacer markers. Dominant bacterial phyla among non-cultured (i.e. native blood) samples were *Proteobacteria* 93%, *Firmicutes* 2%, *Actinobacteria* 2% and *Planctomycetes* 2%, while among cultured samples *Proteobacteria* were 48%, *Firmicutes* 26%, *Actinobacteria* 17%, *Bacteroidetes* 4%, *Cyanobacteria* 3%. Our studies fill a knowledge gap and provide an analysis of the cultivability of circulating blood microbiota. In summary, our findings indicate high biodiversity and distinct mechanisms of propagation of eukaryotic and prokaryotic microbiota inhabiting the blood.

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PP–25

THERAPEUTIC POTENTIAL OF *SATUREJA MONTANA* DERIVED POLYPHENOLS IN THE CENTRAL NERVOUS SYSTEM DISORDERS

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Satureja montana (SM) is recognized as a species of considerable pharmacological significance within the extensive Lamiaceae family, native to the Balkan region. This plant is characterized by a diverse array of secondary metabolites.. Notably, two of its active phenolic compounds, rosmarinic acid and carvacrol, demonstrate significant anxiolytic and antidepressant effects. The objective of the current review was to explore the potential of SM, both as a medicinal herb and a traditional spice, in promoting mental health through its rich array of phenolic compounds. To accomplish the established goal, we gathered and thoroughly examined the most recent scientific literature regarding the neuroprotective properties of SM extracts and their primary polyphenolic constituents. A comprehensive literature review was conducted utilizing the Web of Science, Scopus, PubMed, and ResearchGate databases. Ethnopharmacological studies indicate the potential of SM to influence various stress-related chronic mental disorders, such as anxiety, depression, mild cognitive impairments, and dementia. Research has shown that the dry extract of SM displayed strong anxiolytic and antidepressant properties, outperforming both rosmarinic acid and carvacrol when administered individually [1]. In a comparative study of various species within the Lamiaceae family, SM was identified as one of the plants exhibiting notable acetylcholinesterase inhibitory activity [2]. The inhibition of acetylcholinesterase can improve cholinergic transmission, which is advantageous in conditions such as Alzheimer's disease, where cholinergic deficits are significant. Furthermore, a notable relationship exists between depression, anxiety, and cognitive decline, including dementia. Given that Alzheimer's disease is a complex condition, it necessitates multi-targeted therapeutic approaches for both prevention and management. The primary mechanisms through which *Satureja montana* influences pathological processes in the central nervous system include significant anxiolytic activity, anticholinergic effects, and strong antioxidant and anti-inflammatory properties, which contribute to moderate cognitive benefits. In summary, SM may serve as a valuable and promising alternative in the prevention and treatment of various mental health disorders, including dementia.

Keywords: *Satureja montana*; polyphenols; mental health; Alzheimer's disease.

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PP–26

ELIMINATION OF *CANDIDATUS PHYTOPLASMA SOLANI* FROM *PETUNIA HYBRIDA* VIA *IN VITRO* CULTURE OF APICAL MERISTEMS COMBINED WITH THERMOTHERAPY

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In vitro plant cultures represent a well-established biotechnological platform for microclonal propagation of genetically identical specimens under strictly controlled conditions. Using apical meristems as starting material allows the initiation of pathogen-free cultures because the limited systemic distribution of pathogens in this tissue prevents their spread. *Petunia hybrida* is a widely cultivated ornamental species propagated by both seeds and vegetative cuttings. It is grown under various abiotic conditions and often in proximity to other cultivated or wild plants, increasing the risk of infection by a broad range of phytopathogens, including fungal, bacterial, viral, and phytoplasma agents. *Candidatus Phytoplasma solani* is among the most important pathogens that affect the growth and ornamental value of this species. Due to the high risk of latent or difficult-to-eliminate pathogens such as phytoplasmas in the starting plant material, in vitro techniques based on apical meristems have emerged as an effective strategy for producing healthy and certified planting material.

In the present study, *Petunia hybrida* infected with *Candidatus Phytoplasma solani* was used to initiate in vitro culture. Successful cultivation of apical meristems of Petunia hybrida was achieved through the application of thermotherapy, and the influence of different nutrient media on the regenerative potential of the explants was assessed. These results highlight the effectiveness of combining thermotherapy and apical meristem culture as a reliable method for eliminating *Candidatus Phytoplasma solani* and production of pathogen-free *Petunia hybrida* plants suitable for safe propagation and ornamental use.

Keywords: *Petunia hybrida; Candidatus Phytoplasma solani;* apical meristem tissue culture.

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PP–27

OPTIMIZING MICROPROPAGATION OF *PRUNUS MAHALEB* (L.) ROOTSTOCK IN PLANTFORM™ TIS BIOREACTOR

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Mahaleb cherry (*Prunus mahaleb* L.) is widely recognized as a reliable rootstock for both sweet and sour cherry trees. Noted for its excellent drought and cold tolerance, this species forms a deep, robust root system. Cherry cultivars grafted onto mahaleb rootstock exhibit high graft compatibility, develop expansive crowns, and consistently produce fruit.

The concept of bioreactors for microclonal propagation relies on the transition from semi-solid (agar-based) media to liquid nutrient media, which provides plants with improved access to nutrients, facilitating efficient uptake and enhanced biomass accumulation. Temporary immersion systems (TIS) have gained widespread application, as this approach effectively addresses challenges associated with the high phenolic content in woody species, which often leads to tissue and medium oxidation and markedly reduces explant proliferation.

This study evaluates the large-scale propagation of *P. mahaleb* using the PlantForm™ TIS bioreactor with three different nutrient media, all subjected to the same aeration and medium supply regime. In vitro cultivated shoots were grown in liquid proliferation media, including Murashige and Skoog (MS), McCown Woody Plant Medium (WPM), and DKW/Juglans medium. All media were supplemented with 1 mg/L BAP (6-benzylaminopurine), 0.01 mg/L NAA (α-naphthaleneacetic acid) and 30 g/L sucrose.

Micropropagation success was assessed based on shoot length, number of shoots, number of leaves, callus formation, dry weight, and fresh weight. The results demonstrate that the PlantForm™ TIS bioreactor is an effective system for the large-scale propagation of *P. mahaleb*. Among the three tested media, DKW/Juglans medium proved to be the most suitable, supporting the highest shoot proliferation rate, shoot length, and leaf number, with minimal callus formation. Murashige and Skoog medium also showed good performance, while McCown Woody Plant Medium resulted in reduced shoot multiplication and increased callus production. These findings suggest that DKW medium, combined with the TIS system, provides an optimised approach for the efficient micropropagation of *P. mahaleb*.

Keywords: *Prunus mahaleb* L.; micropropagation; PlantForm™ TIS bioreactor.

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PP–28

ACCUMULATION OF ANTIOXIDANT METABOLITES IN *IN VITRO* PROPAGATED *ARNICA MONTANA* WITH YEAST EXTRACT AND SALICYLIC ACID

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Arnica montana (Asteraceae family) is an herbaceous medicinal plant native to Europe, serving as a source of raw materials rich in secondary metabolites. It has been extensively utilised in the pharmaceutical and cosmetic industries. Due to the manifold intensification of agriculture, overharvesting of plants, and high cattle density in arnica habitats, arnica populations have gradually diminished or disappeared entirely. To conserve *A. montana*, biotechnological approaches for propagation are being employed. However, there is very limited knowledge regarding the impact of treatment with abiotic and biotic elicitors during arnica in vitro propagation on the levels of secondary metabolites possessing antioxidant properties. *A. montana* plantlets were cultivated on MS nutrient medium supplemented with varying concentrations of yeast extract (50, 100, or 200 mg L^{-1} YE) or salicylic acid (50, 100, or 200 µM SA). Both the biotic YE and the abiotic SA elicitors have modulated the levels of metabolites with antioxidant potential against oxidative stress in different ways. The results indicated that YE applied at 100 mg L^{-1} significantly promotes total phenolic and flavonoid content, as well as water-soluble metabolites with antioxidant potential, compared to the untreated control plantlets. Conversely, the addition of salicylic acid to the MS culture medium resulted in a decrease in the accumulation of total phenols and flavonoids, along with water-soluble metabolites possessing antioxidant capacity. As the concentration of SA increases from 50 µM to 200 µM, the extent of decrease in these parameters intensified. Only the lipid-soluble metabolite content increased. *A. montana* plantlets micro-propagated with YE exhibited enhanced levels of total antioxidant capabilities as measured by ferric reducing antioxidant power (FRAP method) and 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity (DPPH method). When plantlets were treated with SA, the free radical scavenging activity remained unchanged, and the FRAP measured in the *A. montana* plantlets was significantly decreased compared with untreated control plants. Overall, the results of this study revealed that the presence of biotic YE and abiotic SA elicitors in the MS nutrient medium alters the biosynthesis of antioxidant metabolites in *A. montana* plantlets, depending on the type and concentration of the elicitor.

Keywords: *Arnica montana*; yest extract; salicylic acid; *in vitro* propagation; secondary metabolites.

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PP–29

CHANGES IN BIOMETRICS AND ACTIVITY OF ANTIOXIDANT ENZYMES IN *IN VITRO* CULTIVATED *ARNICA MONTANA* AFTER ELICITATION WITH METHYL JASMONATE

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Arnica montana L. is a valuable medicinal plant with strong anti-inflammatory properties due to its rich content of antioxidants and specific secondary metabolites. The species is a European endemic, whose natural populations are threatened due to habitat disturbance and poor management. *In vitro* plant cultures are an alternative method for rapid plant multiplication and controlled synthesis of biologically active substances. Elicitation is a common strategy to induce physiological changes and defense responses in plants, thereby increasing their antioxidant potential and the synthesis of specific secondary metabolites. Methyl jasmonate (MeJA), as a plant growth regulator that modulates plant growth and development from morphological to molecular level is increasingly used as an elicitor. This study aims to assess the growth and antioxidant enzyme activity of *in vitro* cultivated *A. montana* after 3- and 7-day treatment with different concentrations of MeJA (0, 50, 100 and 200 µM). Regarding biometric parameters (number of shoots per explant, plant height and fresh weight) no statistically significant differences were observed on the 3d day of treatment except the highest concentration of MeJA (200 µM) that led to plant height reduction. All three biometric parameters decreased significantly when arnica was cultivated in the presence of MeJA for seven days. Regarding the activity of the main antioxidant enzymes, three different trends were observed: superoxide dismutase activity increased with increasing MeJA concentration and treatment duration; catalase and ascorbate peroxidase activities were highest at 100 µM MeJA on the 3rd day of treatment; guaiacol peroxidase activity was negatively affected by MeJA. Total antioxidant activity measured by ferric-reducing antioxidant power (FRAP) increased after MeJA treatment at all tested concentrations, with it being higher on the seventh day compared to the third day. The results showed that MeJA affects biometric parameters and antioxidant enzyme activity differently depending on the concentration and duration of treatment.

Keywords: *Arnica montana*; *in vitro* multiplication; methyl jasmonate; antioxidant enzymes.

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PP–30

ACCUMULATION OF PHENOLIC COMPOUNDS IN *ARNICA MONTANA IN VITRO* PLANTLETS AFTER TREATMENT WITH METHYL JASMONATE

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Arnica montana L. (mountain tobacco) is a valuable plant species, used for centuries to treat various diseases due to its anti-inflammatory properties. The plant is a rich source of numerous phytochemicals, including sesquiterpene lactones, helenalin, 11α, 13-dihydrohelenalin and their esters, phenols, flavonoids, and essential oils. *A. montana* is a European endemic species whose natural populations are threatened due to disturbance and poor management of the habitats in which the species occurs. Plant cell and tissue cultures can be an alternative tool for rapid and sustainable synthesis of bioactive compounds under precisely controlled conditions. Optimizing cultivation conditions without genetic manipulation reliably increases the synthesis of secondary metabolites. Methyl jasmonate (MeJA) is frequently used for the enhancement of secondary metabolites in *in vitro* culture. This study aimed to assess the content of *A. montana* phenolic compounds after treatment with MeJA at concentrations of 50, 100, or 200 µM for 3 and 7 days. The *in vitro* plants were developed on MS nutrient medium supplemented with 0.5 mg/l BAP. The application of MeJA enhanced the total phenolic and flavonoid content, the production of water- and lipid-soluble metabolites with antioxidant potential, and the accumulation of caffeoylquinic acids. The highest total phenolic content was established in *in vitro* plants treated with 100 µM MeJA for 7 days, showing an almost twofold increase, compared to the control plants. The maximum total flavonoid content was achieved in *in vitro* plants exposed to 50 µm MeJA for 7 days. Treatment with 100 µM MeJA for 3 days was effective for the production of water- and lipid-soluble metabolites. The accumulation of caffeoylquinic acids determined by HPLC was the highest in shoots harvested on the 7th day after treatment with 50 µM MeJA compared to control, untreated shoots. The analysis of the elicitor induced transcript changes of the *PAL* and *HQT* genes revealed that the expression of the two genes is regulated by the treatment with MeJA, irrespective of the elicitor concentration used.

The obtained results showed that MeJA increased the production of phenolic compounds in *in vitro* propagated plants.

Keywords: Arnica; *in vitro* shoots; caffeoylquinic acids; total phenolic and flavonoid content; genes.

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PP–31

IN VITRO CULTIVATION OF MEDICINAL PLANTS AS A TOOL FOR BIODIVERSITY CONSERVATION AND BIOLOGICALLY ACTIVE SUBSTANCES PRODUCTION

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Conservation of biodiversity is of global importance for sustaining ecosystems and preserving natural resources. Land-use change and urban expansion have significantly affected natural ecosystems. Recent studies show that a significant proportion of the world's plant species are at risk of becoming threatened, and 50% of these plants are endemic. *In vitro* cultivation, or plant tissue culture, provides a powerful tool for conserving biodiversity and enhancing the production of valuable bioactive compounds from medicinal plants. By cultivating plant cells, tissues, or organs in controlled laboratory conditions, it becomes possible to propagate plants rapidly, conserve genetic resources, and even modulate the production of specific medicinal compounds. This approach can reduce pressure on wild populations, ensuring sustainable access to medicinal resources while also providing a platform for the discovery and production of novel substances.

Species such as *Clinopodium vulgare* (wild basil), *Sideritis scardica* (Pirin mountain tea), *Marrubium vulgare* (horehound), and *Salvia aethiopis* (African sage) are recognized for their valuable medicinal properties and all have a history of use in traditional medicine for various ailments. Due to poor reproduction in nature and overharvesting, their natural habitats have been depleted. The development of an effective micropropagation system (identical plants) is a way to preserve the gene pool, and the obtained plants can be used as a source of pharmaceutically valuable metabolites. Protocols for efficient clonal in vitro micropropagation of these valuable plants have been established with a prolonged ability to develop in vitro while retaining their morphogenetic potential. The *in vitro* obtained plants are successfully adapted on the experimental fields with a high survival rate. The developed in vitro micropropagation systems provide the opportunity to obtain high-quality planting material for *ex situ* cultivation. The propagated plants demonstrated high phenolic and flavonoid content, along with significant antioxidant activity. Extracts derived from these plants exhibited a dose- and time-dependent inhibitory effect on cell viability and proliferation of human cell lines from breast, cervical, and colorectal carcinomas. While these preliminary findings suggest potential anticancer applications, additional studies are required to confirm their therapeutic efficacy. In summary, biotechnological tools like *in vitro* culture offer a valuable alternative for plant diversity studies and genetic resource conservation. Cultivating these plants in controlled environments helps reduce our dependence on wild populations, contributing to biodiversity conservation while also ensuring a sustainable source of valuable medicinal compounds.

Keywords: *In vitro* propagation; medicinal plants; antioxidant; antitumor activity.

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PP–32

METABARCODING HONEY TO ASSESS PATTERNS OF HONEYBEE PLANT FORAGING PREFERENCES ALONG AN URBANIZATION AND LANDSCAPE GRADIENT

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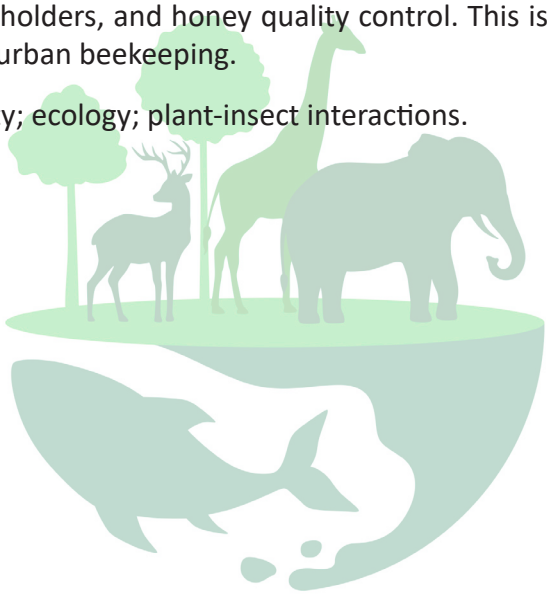
Honey is a natural product derived from honeybee activity and serves as a biological reservoir containing traces of plants, fungi, and bacteria. It thus acts as a bioindicator of biodiversity and ecological interactions among species. These dynamics are often influenced by the eco-geographical landscape. Urbanization is an important factor affecting plant diversity and pollution levels, with ultimate impact on honeybee health, behavior, and foraging preferences. Urbanization often leads to reduced native plant diversity and the expansion of non-native and invasive species, with a predominance of ornamental species and monocultures. Empirical data on the impact of urbanization on the biological content of honey remain limited and sometimes contractionary.

This study aimed to investigate the variations in the botanical composition of honey collected from apiaries in areas with variable level of urbanization – urban, peri-urban and non-urbanized ("wild") ones in Sofia region. We asked two key questions: 1.) How does urbanization affect plant diversity (and thus honeybee`s nutrition resources) and how is this reflected in honeybee foraging preferences? 2.) What is the resolution capacity of metabarcoding for identifying the botanical composition of honey?

Sixty honey samples from 11 apiaries, collected during two seasonal samplings in year 2024, were analyzed using metabarcoding with Illumina NovaSeq sequencing and plant-specific primers targeting the ITS region. We present data showing that the metabarcoding approach offers significantly higher resolution in terms of the number of taxa detected and the minimum detection threshold (%), compared to classical botanical analysis based on pollen morphology. We also present data on beta diversity dynamics as influenced by urbanization levels, along with emerging patterns in plant-insect interactions.

This pioneering study in Bulgaria demonstrates the utility and efficiency of honey metabarcoding as an underutilized biomonitoring tool with potential applications in large-scale biodiversity studies, ecological reporting for stakeholders, and honey quality control. This is particularly relevant in the context of rapidly expanding urban beekeeping.

Keywords: Honey; biodiversity; ecology; plant-insect interactions.



PP–33

EVOLUTIONARY PATTERNS IN THE GENUS *SYMPHYOTRICHUM* IN EAST-CENTRAL EUROPE ASSESSED BY DNA BARCODING

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This study explores the genetic diversity and haplotype distribution of *Symphyotrichum* species, hybrids, and cultivars with varying ploidy levels. It addresses the challenges posed by polyploidization and hybridization, which are known to shape the evolutionary history of the genus.

A total of 157 samples, collected across East-Central Europe - from Austria to Bulgaria, primarily in the Danube basin and its tributaries - were analyzed using DNA barcoding targeting the nuclear ITS and chloroplast *trnH-psbA* regions. The main highlights of our study are:

- DNA barcoding revealed distinct *ITS* and *trnH-psbA* haplotypes, as well as genetic patterns, that vary across the ploidy levels of the studied *Symphyotrichum* species.

- Large-flowered asters in Europe likely arose through the hybridization of multiple species and have a polyphyletic origin

- High intrapopulation but low interpopulation genetic diversity observed in the Danube River basin with rare haplotypes in this region

- New insights into the genetic structure of invasive *Symphyotrichum lanceolatum*, its hybrids (S. × salignum, S. × versicolor), and cultivated forms (S. novi-belgii × dumosum).

Our data highlight the complex evolutionary history of *Symphyotrichum* and underline the need to integrate DNA barcoding with morphometric and karyological analyses for accurate species identification and a reliable assessment of evolutionary patterns.

Keywords: Aster novi-belgii agg.; DNA barcoding; Biological invasions; Hybrid species; Molecular phylogeny.

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PP–34

SOIL OXIDATION-REDUCTION POTENTIAL AND MICROBIAL BIODIVERSITY OF MULCHED *SOLANUM TUBEROSUM* AND *PHASEOLUS VULGARIS*

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The soil oxidation-reduction potential (ORP) is a physico-chemical parameter essential for every soil type as it affects the mobility of mineral nutrients and thus the physiological activity of both soil microbiota and plants. The soil organisms and the plant roots exudates reversibly affect the ORP fluctuation within the soil substrate. This study is focused on the correlation between the soil ORP values, soil microbial biodiversity and plant productivity. The investigation included an agricultural field setup of two model crops – *Solanum tuberosum* and *Phaseolus vulgaris*, cultivated with and without the application of soil-covering mulch. Depending on the plant species, the measured soil ORP values varied from the control setups without plants. Moreover, a strong positive correlation was established between mulching and the soil ORP and microbial biodiversity. Accordingly, in the mulched setup, the yield of *S. tuberosum* increased noticeably, while the effect on *P. vulgaris* did not change significantly. The quality of potato tubers was affected by mulching, which led to a decrease in phenolic compounds and an increase in soluble sugars and starch levels. In beans, both amino acid and sugar contents also increased. The overall data suggested that mulching ameliorates both the soil state and crop yield.

Keywords: Bean; mulching; potato tuber; soil microbiome; soil physico-chemistry; yield.

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PP–35

APPLICATION OF DNA BARCODING FOR STUDYING THE TAXONOMY OF BULGARIAN WHITE OAKS (*SUBGENUS QUERCUS*)

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The genus *Quercus* in Bulgaria is characterized by high phenotypic diversity, which has resulted in identification and description of numerous taxa with different status. Still there are a lot of unresolved problems and issues related to the systematic relationships among the taxa and their position within the framework of the existing classification schemes. We applied DNA barcoding approach to solve the taxonomic puzzle. Eight taxa, considered as distinct species of white oaks were included in the study, namely *Quercus robur*, *Q. pedunculiflora*, *Q. mestensis*, *Q. petraea*, *Q. hartwissiana*, *Q. protoroburoides*, *Q. frainetto*, and *Q. pubescens*. DNA barcoding based on one nuclear (ITS) and two chloroplast markers (*trnH-psbA*, *matK*) proved promising but not sufficient, as none of the markers proved to be species-specific. However, some combinations of the markers could successfully identify three of the species. The applied markers allowed a good estimate of variation among the groups of species and groups of populations. Results of the study outlined the necessity of development of new markers and approaches to resolve the taxonomic puzzle of this taxonomically very complex group.

Keywords: Oaks; taxonomy; DNA barcoding; markers.

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AFTERWORD

Closing Remarks

The First International Conference on Functional Biology brings together researchers, students and professionals from many fields to share knowledge, exchange ideas and inspire new collaborations. The contributions collected in this Book of Abstracts show not only the richness of functional biology today but also its potential to address global challenges such as climate change, food security and sustainable development in the future.

We thank all participants, speakers and partners for their commitment and enthusiasm. We look forward to seeing the seeds of ideas planted here in Varna grow into fruitful collaborations, innovative projects and lasting friendships.

See you at the next edition of the Functional Biology Conference!