

**The Conference –
100th Anniversary of the Department of Plant Physiology
Warsaw University of Life Sciences
“Plant Physiology in the 21st century”**

**Konferencja –
100-lecie Katedry Fizjologii Roślin
Szkoły Głównej Gospodarstwa Wiejskiego w Warszawie
“Fizjologia roślin w XXI wieku”**

15-16th September 2022’

15-16 września 2022’

Abstracts

Abstrakty



Warsaw, Poland

Applications in the use of metabolic profiling in the study of plant interactions – a focus on allelopathy and interference

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Plant secondary products are the drivers of many plant interactions, both above and below ground, with other plants, herbivores and soil-borne macro- and microbiota. During plant growth and cellular ontogeny, secondary plant products are biosynthesized and often accumulate in a highly regulated manner in specialized tissues and organelles. Metabolites released by plants and their residues play important roles in rhizosphere signalling, plant defence and responses to abiotic stresses. Plants employ a variety of sequestration and transport mechanisms to store and release bioactive metabolites and export them selectively into the surrounding environment. Transport systems in plant roots and shoots are generally closely linked to soil environmental conditions and plant health. Release of key metabolites often involves enzymatic activity leading to tissue degradation, but is also achieved by active transport, simple diffusion, extrusion or active exudation. In actively growing roots, specialized cells generate and release large quantities of allelochemicals in response to stressors in local environments.

Our research has focused on the production, localization and release of plant-produced metabolites by combining fluorescence and confocal microscopic observations with metabolic profiling. We have employed both targeted and non-targeted metabolomics to identify novel metabolites and pathways in response to plant-plant and plant-microbe (resulting in symbiosis, allelopathy and/or phytotoxicity) and plant-grazing herbivore interactions (resulting in photocytotoxicity/poisoning). By identification of bioactive metabolites of interest, the regulation of biosynthetic pathways in plants can be studied, as well as their microbial or chemical transformation products. Here we present examples of metabolites playing key roles in crop interference with weeds, invasion success of exotic weeds, transformation processes leading to bioherbicide formation, livestock poisoning, reproductive failure and photosensitization. Opportunities for bioprospecting of novel metabolites as lead molecules for biologically-based crop protection are also discussed.

Keywords: Metabolomics, secondary plant products, photosensitization, phytotoxicity, allelopathy, plant defense

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An overview of the role of ROS in seed biology: advances and prospects

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Reactive oxygen species (ROS) have been reported to play a pivotal role in the regulation of seed germination and dormancy. Although their mode of action is still far from being fully understood, major progresses have been made this last decade that open novel fields of investigation. Here we will sum-up the findings recently obtained that shed a new light on the signalling roles of ROS in seed biology. We will show that seed imbibition and germination are associated with a timely and spatially controlled generation of ROS, and that ROS homeostasis plays a key role in the perception and transduction of environmental conditions controlling germination. Our recent findings also highlight the key role of ROS trafficking between organelles and cell compartments in cell signalling during seed germination. We will focus on the role of mitochondria, as an environmental and developmental sensor organelle that regulates ROS production, and on downstream events, such as nuclear redox status and chromatin oxidation, in the completion of seed germination.

Oxygen, a key factor in energy metabolism and hormone signaling pathways involved in seed germination and dormancy regulation

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I am honored to be invited to participate the 100th Anniversary of the establishment of the Department of Plant Physiology of the Institute of Biology at Warsaw University of Life Sciences. Oxygen is a major factor of seed germination since it allows resumption of respiration and subsequent metabolism reactivation during seed imbibition, thus leading to the production of reducing power and regulating the energy charge. Physiological studies carried out in the 70's - 80's indicate that oxygen requirement depends on the species and is modulated by the environmental factors. They have also demonstrated that the covering structures mainly inhibit germination by limiting oxygen supply to the embryo during imbibition through enzymatic oxidation of phenolic compounds by polyphenol oxidases. Here I will also highlight the major data obtained over the last 20 years indicating the key role of oxygen in the cellular and molecular networks regulating seed germination and dormancy, through the hormone signaling pathways including ABA, GAs and ethylene metabolism and sensitivity, and the emerging role of mitochondria in ROS production.

I dedicate this lecture to my friend Prof. Renata Bogatek, with whom I collaborated during more than 30 years, first when she was young researcher in the Institute of Botany at the University of Warsaw, and second from 2001 when she was assistant Professor and then head of Chair of Plant Physiology in 2003 at the Warsaw Agricultural University. She was a well known scientist working on the role of metabolism and ROS in seed dormancy.

Functions of nitric oxide (NO) and hydrogen sulfide (H₂S) in higher plants

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The physiology and biochemistry of higher plants have undergone a particular paradigm regarding two gaseous molecules, nitric oxide (NO) and hydrogen sulfide (H₂S), considered previously toxic by the scientific community. Nowadays, accumulating data have shown that both molecules possess signaling functions and participate in almost all the physiological processes (seed germination, growth and development, stomatal movement, senescence, flowering, and fruit ripening) as well as in the mechanism of response against abiotic and abiotic stresses. At the biochemical level, NO as H₂S directly or indirectly exert its function through post-translational modifications (PTMs) of proteins, mainly S-nitrosation and persulfidation because they operate as cysteine-based redox switches. Furthermore, NO and H₂S could have biotechnological applications because when they are exogenously applied they exert beneficial effects against different types of stresses (salinity, heavy metals, etc.) and preserve the fruits during postharvest storage since, in many cases, NO and H₂S can regulate the metabolism of reactive oxygen species (ROS) through the modulation of the antioxidant systems.

Funding: [Ministry of Science and Innovation (PID2019-103924GB-I00) and Junta de Andalucía (P18-FR-1359), Spain]

The Plant Mitochondrial Proteome

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Plant mitochondria perform a wide range of functions in the plant cell ranging from providing energy and metabolic intermediates, via coenzyme biosynthesis and their own biogenesis to retrograde signaling and programmed cell death. To perform these functions, they contain a proteome of >2000 different proteins expressed in some cells under some conditions. The vast majority of these proteins are imported, in most cases by a dedicated protein import machinery. Recent proteomic studies have identified 2500-3000 different proteins in each of Arabidopsis, bean and potato mitochondria, no doubt including some contaminants. The largest groups are energy-related proteins, membrane transporters, and RNA-associated proteins such as PPR proteins. The first two groups are present in high copy numbers while the RNA-associated proteins and other proteins are present in low copy numbers even substoichiometric, pointing at a heterogeneous condriome. The proteomic studies have improved our understanding of basic mitochondrial functions, have led to the discovery of new mitochondrial metabolic pathways and are helping us towards appreciating the dynamic role of the mitochondria in the responses of the plant cell to biotic and abiotic stress.

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Structure-based ligand design and discovery of novel tenuazonic acid derivatives with high herbicidal activity

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Tenuazonic acid (TeA), a typical representative of the natural tetramic acid family, has been recognized as a potential bioherbicide. However, its herbicidal efficacy is still not up to the ideal standard of commercial products. In this research, structure-based ligand design was used to find new TeA's derivatives with improved potency. With the aid of a constructed molecular model of natural lead molecule TeA binding to the Q_B site in *Arabidopsis* D1 protein, a series of derivatives differing in the alkyl side chain were designed and ranked according to free energies. We analyzed the binding model of each derivative inside the Q_B site, and found that all of them are stabilized by hydrogen bonding interactions between their carbonyl oxygen O2 and D1-Gly256 residue; moreover, hydrogen bond distance seems to be the most important factor for maintaining high binding affinity. Based on their better binding affinities compared to that of TeA, derivatives designated as D6, D13 and D27 were synthesized to evaluate their photosynthetic inhibitory activity and herbicidal efficacy. D6 and D13 with sec-pentyl and sec-hexyl side chains, respectively, were about twice more inhibitory of oxygen evolution rate and double higher herbicidal efficacy than TeA with a sec-butyl side chain. Simulated docking of TeA and the three derivatives to the Q_B site of three different plants showed that there is a significant linear relationship between herbicidal efficacy and binding affinity and hydrogen bond distance. These findings indicate that D6 and D13 are promising compounds to develop TeA-derived novel PSII herbicides with superior performance.

Keywords: Photosynthetic inhibitor; D1 protein; Docking; Homology modeling

Metabolic changes induced by *Cuscuta campestris* Yunck on the host species *Artemisia campestris* subsp. *variabilis* (Ten.) Greuter as a strategy for successful parasitization

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Cuscuta campestris is a holoparasitic species that parasitises several wild species and crops, resulting in drastically reduced crop yields. Among its hosts, *Artemisia campestris* is a species of pharmaceutical and nutraceutical interest and one of the most affected in natural ecosystems. There is limited information about the mechanism of host recognition; in addition, there are no available data on the interactions of *Cuscuta* and *A. campestris* and the effects on the primary and specialized metabolic processes of host plants during or after the parasitization event. This investigation focused on the composition of volatile organic compounds (VOCs) involved in host-parasite interactions and associated metabolomics scale changes in primary metabolism and several physiological parameters of the host. VOCs' analysis showed a reduction in the levels of sesquiterpenoids and an increase in monoterpenoids; metabolites involved in plant defense and host recognition, respectively. Moreover, *Cuscuta* reduced 3hexenyl-acetate levels in the host, a metabolite with known repellent activity against *Cuscuta* itself, and altered plant hydration status, forcing stomatal opening and stimulating plant transpiration. Consequently, *Cuscuta* parasitization induced indirect physical damage to the PSII, thus reducing the efficiency of the photosynthetic machinery of the host. Finally, an untargeted-metabolomics analysis highlighted that the parasitization perturbed the amino acids and glycogen metabolism, increasing the production of osmoprotectants. In conclusion, we hypothesize that *Cuscuta* parasitization increases internal host defenses (via primary metabolites fundamental for plant survival) at the expense of environmentally directed ones (via specialized metabolites) in the host species *Artemisia*, thus limiting plant defense against progressive parasitization.

Chlorophyll fluorescence studies between Poland and Italy

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I am professor of Forest Botany at the University of Firenze (Italy), and my research interests include forest ecology and the responses of trees to environmental stress and climate change. My research deals with the developing of rapid, non-invasive physiological tests to assess the conditions of trees in field conditions. For this reason, I developed a thigh collaboration with the laboratory of prof. Reto J. Strasser, in the University of Geneva (Switzerland). Within this collaboration, I knew Hazem M. Kalaji of the Warsaw University of Life Science (Poland) during a plant physiology congress at Krakow (Poland) and, since the begin of the XXI century, I begun to collaborate with him within the chlorophyll fluorescence (ChlF) studies and application. Our collaboration has materialized in academic exchanges between our respective institutions: I had some talks in Warsaw, and Hazem M. Kalaji in Florence. Moreover, we produced joint publications, among them a practical booklet, in national language, on chlorophyll fluorescence principles and applications, and two comprehensive reviews on ChlF in the scientific journal "Photosynthesis Research". We participated in editorial scientific initiatives organized respectively by me and Hazem. The collaboration with Hazem M. Kalaji and the Warsaw University of Life Science was always fruitful and exciting e certainly that will be enhanced and widened in the future with the involvement of more colleagues and collaborators.

Reproductive biology in the olive tree: physiological, agronomical and biomedical implications

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The olive tree is a crop of paramount importance for the economy of Mediterranean countries, and a model to analyze numerous aspects of plant reproductive biology by our research group. Floral induction, pollen and pistil development and interaction, pollen tube growth and fertilization and fruit development are events heavily involved in the olive yield, and therefore, topics of our studies. Our analyses are highly multidisciplinary and involve the generation of reproductive transcriptomes, which are the basis of biochemical and molecular validations of the expression of highly informative genes revealing key functions in the physiology of the reproduction. Thus, we are mainly unraveling the role of ROS and NO as well as lipid metabolism in olive reproduction. Many of these studies have been carried out in collaboration with Polish researchers. Moreover, our analyses are tightly connected with the development of agronomical practices for this plant, the characterization of the numerous olive varieties present over the world, and the design of methods for quality control applicable to olive oils, as well as proposing alternative uses for by-products of the olive, jointly with the olive oil and table olive producing industry. Finally, we also explore the presence in the olive reproductive tissues of important molecules which, in addition to their physiological significance for the plant, interact with humans in either positive (as nutrients, nutraceuticals, anti-inflammatory, anti-diabetic...), or negative (allergens) aspects.

This research was funded by research projects MCIN/AEI/PID2020-113324GB-I00, JA/PAIDI/P18-RT-1577, JA/PAIDI/PYC20 RE009CSIC.EEZ y JA/PAIDI/ UMA20-FEDERJA-029, all of them co-funded by ERDF program of the EU.

Nitric oxide – a multitask player in plant interactions with pathogenic fungi and oomycetes

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Nitric oxide (NO) has been recognized as a key signalling and regulatory molecule in multiple organisms. NO and reactive nitrogen species (RNS) participate in diverse processes during growth, development, reproduction, and responses to the external environment, including biotic interactions. NO has been shown to form a component of signalling pathways and defence responses in multiple forms of plant interactions with their microbial counterparts, ranging from symbiotic to pathogenic modes. This talk presents current knowledge on the functions of NO in plant responses to biotrophic, necrotrophic, and hemibiotrophic fungi and oomycetes. Our previous studies revealed a decisive role of time- and site-specific NO production in germination, oriented growth, and active penetration of the host tissues by filamentous pathogens, as well as in pathogen recognition and defence activation in plant tissues. Increased levels of NO and RNS during early pathogen recognition are involved in the initiation of defence mechanisms, which in the latter stages result in the inhibition of pathogen growth in resistant genotypes. NO signalling pathways are involved in the regulation of the fungal pathogen growth, namely in the early stages of conidia germination and the development of germination tubes. In the latter stages of pathogenesis associated with the formation of appressoria and penetration of fungal structures, NO and RNS act as effector molecules in the mechanism of hypersensitive response and initiation of further defence within signalling pathways of plant hormones. Modulations of RNS and enzymes of their metabolism are also involved in the systemic defence activation in distant non-infected plant tissues. Collectively, NO in interplay with reactive oxygen species governs successful plant colonization, cell death or establishment of resistance to diverse fungal and oomycete phytopathogens.

Proteogenomic and redox proteomic analysis of seed dormancy reveals phenotype- and genotype-associated signatures of pre-harvest sprouting resistance in hexaploid wheat

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Control of pre-harvest sprouting resistance in wheat is complex, genetic factors responsible for it are dispersed on almost every wheat chromosome, and interactions occur between QTLs and environment. In this study, proteomic signatures of pre-harvest sprouting resistance were analyzed using lines with marginal dormancy phenotypes derived from a hard white spring wheat (*Triticum aestivum* L.) doubled haploid population, iTRAQ- and iodoTMT-based proteomics integrated with customized EST and reference wheat genome databases, RNA-Seq analysis, and association with QTL regions. Altogether 7776 unique proteins were identified with high confidence, of which 78 proteins showed significant differential abundance in embryos isolated from seeds with dormant and non-dormant phenotypes. Changes in abundance of 449 unique embryo proteins were dormancy genotype-specific. In dormant embryos, phenotype-specific alterations were found for proteins involved in modulation of reactive oxygen species in cell wall, extracellular compartment, and mitochondria, and regulation of programmed cell death. Proteomic signatures of dormancy phenotype consisted of signaling proteins associated with flowering, downstream targets of ABA-dependent germination repression pathway, enzymes responsible for alteration of intracellular ABA levels, structural integrity and physicochemical properties of plant cell wall. In embryos with non-dormant phenotype, energy metabolism showed high capacity for the provision of NADPH reducing equivalents, pyruvate and TCA cycle intermediates for biosynthetic processes, and cytosolic redox control. Induction of early germination in embryos was accompanied by higher capacity for generation of lipid second messengers, protein targeting to 26S proteasome, positive modulation of GA signaling, and regulation of osmosensory responses.

Keywords: After-ripening, dormancy, embryo, proteogenomics, seed, *Triticum aestivum*

Arabidopsis root apical meristem survival during hypoxia-induced waterlogging is determined by phytoglobin through nitric oxide and auxin

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Plant performance to waterlogging is ameliorated by the over-expression of the *Arabidopsis Phytoglobin 1 (Pgb1)* which also contributes to the maintenance of a functional RAM. Hypoxia induces accumulation of ROS and damage in roots of wild type plants; these events were preceded by the exhaustion of the RAM resulting from the loss of functionality of the *WOX5*-expressing quiescent cells (QCs). These phenotypic deviations were exacerbated by suppression of *Pgb1* and attenuated when the same gene was up-regulated. Genetic and pharmacological studies demonstrated that degradation of the RAM in hypoxic roots is attributed to a reduction in the auxin maximum at the root tip, necessary for the specification of the QC. This reduction was primarily caused by alterations in PIN-mediated auxin flow but not auxin synthesis. The expression and localization patterns of several PINs, including PIN1, 2, 3 and 4, facilitating the basipetal translocation of auxin and its distribution at the root tip, were altered in hypoxic WT and *Pgb1*-suppressing roots but mostly unchanged in those over-expressing *Pgb1*. Disruption of PIN1 and PIN2 signal in hypoxic roots suppressing *Pgb1* initiated in the transition zone at 12h and was specifically associated to the absence of *Pgb1* protein in the same region. Exogenous auxin restored a functional RAM while inhibition of the directional auxin flow exacerbated the degradation of the RAM. The regulation of root behavior by *Pgb1* was mediated by nitric oxide (NO) in a model consistent with the recognized function of Pgbs as NO scavengers. Collectively, this study contributes to our understanding of the role of Pgbs in preserving root meristem function and QC niche during conditions of stress, and suggests that the root transition zone is most vulnerable to the stress.