Appendix no 10

Summary of Professional Accomplishments

Identification of metabolomic markers in plants from family *Poaceae* and *Brassicaceae* related to response to abiotic and biotic stress

Anna Piasecka, PhD
Institute of Bioorganic Chemistry
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Poznań 2024

1. **Name:** Anna Piasecka

2. **Degrees and Academic Titles**

• **2013**: Doctor of Agricultural Sciences (with honor)

Institute of Plant Genetics, Polish Academy of Sciences in Poznan

Doctoral thesis on "Changes in phenolic profiles of secondary metabolites in barley plants (*Hordeum vulgare* L.) subjected to water stress".

Supervisor: Prof. Piotr Kachlicki

• **2009**: Master of Biotechnology (with honor)

Adam Mickiewicz University in Poznan

Master's thesis on "Characterization of superoxide dismutases in selected *Brassica* species".

Supervisor: Prof. dr hab. Jan Sadowski

• **2009**: Engineer in Food Technology and Human Nutrition

Poznan University of Life Sciences, Faculty of Food Science and Human Nutrition

Bachelor's thesis on "Microencapsulation of proteins - methods and applications".

Supervisor: Dr. hab. Kamila Goderska.

3. **Information about previous employment in scientific institutions:**

• **2/2016** - present: Assistant Professor,

Department of Functional Plant Metabolomics, Institute of Bioorganic Chemistry, Polish Academy of Sciences in Poznań

• **1/2015 - 09/2020**: Senior Specialist,

Metabolomics Team, Institute of Plant Genetics, Polish Academy of Sciences

• **1/2011- 12/2014**: Research Assistant,

Metabolomics Team, Institute of Plant Genetics, Polish Academy of Sciences

• **10/2008-12/2010**: PhD student,

Metabolomics Team, Institute of Plant Genetics, Polish Academy of Sciences

• Maternity and parental leaves: **2/2010-8/2010** and **3/2015-2/2016**.

4. **Description of the achievements, referred to in Article 219(1)(2) of the Act of 20 July 2018 on Higher Education and Science (Journal of Laws of 2021, item 478, as amended)**

4.1. Title of the scientific achievement

Identification of metabolomic markers in plants from family Poaceae and Brassicaceae related to response to abiotic and biotic stress

4.2. Publications comprising the scientific achievement

H1. Piasecka A[#], Sawikowska A[#], Kuczyńska A, Ogrodowicz P, Mikołajczak K, Krystkowiak K, Gudyś K, Guzy-Wróbelska J, Krajewski P, Kachlicki P*. (2017). Drought-related secondary metabolites of barley (*Hordeum vulgare* L.) leaves and their association with mQTLs. *Plant Journal* 2017; 89: 898–913; https://doi.org/10.1111/tpj.13430

F_{2017}= 5.901; $MNiSW_{2017}$=45; $MNiSW_{2024}$= 140; C=67

My contribution to the creation of this publication consisted of: formulating the research problem and concept of the work (together with the corresponding author), planning and conducting all metabolomic experiments, analyzing the obtained data, interpreting the data (together with the corresponding author), writing the first draft of the manuscript in its entirety, editing and improving the final version of the manuscript (together with the corresponding author).

H2. Piasecka A, Sawikowska A, Kuczyńska A, Ogrodowicz P, Mikołajczak K, Krajewski P, Kachlicki P*. Phenolic Metabolites from Barley in Contribution to Phenome in soil Moisture Deficit. *International Journal of Molecular Sciences* 2020; 21(17): 6032. https://doi.org/10.3390/ijms21176032;

IF_{2019}=4.556; $MNiSW_{2019}$=140 pkt; $MNiSW_{2024}$=140 pkt; C=4

My contribution to the creation of this publication consisted of: formulating the research problem and concept of the work (together with the corresponding author), planning and conducting all metabolomic experiments, analyzing the obtained data, interpreting the data (together with the corresponding author), writing the first draft of the manuscript in its entirety,

editing and improving the final version of the manuscript (together with the corresponding author).

H3. Sawikowska A, **Piasecka A**, Kachlicki P, Krajewski P*. Separation of Chromatographic Co-Eluted Compounds by Clustering and by Functional Data Analysis. *Metabolites 2021* 11(4): 214; https://doi.org/10.3390/metabo11040214

IF_{2020}=4.097; $MNiSW_{2020}$=100 pkt; $MNiSW_{2024}$=100 pkt; C=2

My contribution to the creation of the publication consisted of: planning and conducting experiments, analyzing the obtained data, interpreting the obtained data (together with the first author and corresponding author), collecting and analyzing source materials and developing them with the other co-authors, writing part of the first version of the manuscript, editing the final version of the manuscript and its correction in response to reviews (together with the corresponding author).

H4. Piasecka A, Kachlicki P, Stobiecki M*. Analytical Methods for Detection of Plant Metabolomes Changes in Response to Biotic and Abiotic Stresses. *International Journal of Molecular Sciences* 2019; 20(2): 379; https://doi.org/10.3390/ijms20020379

IF_{2018}=4.183; $MNiSW_{2018}$=30 pkt; $MNiSW_{2024}$=140 pkt; C=60

My contribution to the creation of the publication consisted of: formulating the research problem and work concept (together with the corresponding author), collecting and analyzing source materials and developing them with the other co-authors, writing part of the first version of the manuscript, preparing illustrations, editing and correcting the final version of the manuscript (together with the corresponding author).

H5. Piasecka A*, Sawikowska A, Witaszak N, Waśkiewicz A; Kańczurzewska M; Kaczmarek J; Lalak-Kańczugowska J. Metabolomic Aspects of Conservative and Resistance-related Elements of Response to *Fusarium culmorum* in the Grass Family. *Cells* 2022; *11*(20): 3213; https://doi.org/10.3390/cells11203213

F_{2021}= 7.666; $MNiSW_{2021}$=140 pkt; $MNiSW_{2024}$=140 pkt; C=1

My contribution to the publication involved formulating the research problem and the concept of the work, planning the experiments, developing the research methodology, performing the experiments, analyzing the data, interpreting the data, writing the first draft of

the manuscript, and full editing and revising of the manuscript in response to the reviewers' comments.

H6. Piasecka A*, Sawikowska A, Jedrzejczak-Rey N, Piślewska-Bednarek M, Bednarek P. Targeted and Untargeted Metabolomic Analyses Reveal Organ Specificity of Specialized Metabolites in the Model Grass *Brachypodium distachyon*. *Molecules* 2022; 27(18): 5956. https://doi.org/10.3390/molecules27185956

IF_{2021}= 4.927; $MNiSW_{2021}$=100 pkt; $MNiSW_{2024}$=140 pkt; C=1

My contribution to the creation of the publications consisted of: formulating the research problem and work concept, participating in the development of the research methodology, overseeing experiments, conducting experiments, analyzing the obtained data, interpreting the data (together with the corresponding author), writing the first version of the manuscript, editing and correcting the final version of the manuscript (together with the corresponding author).

H7. Winkelmüller TM, Entila F, Anver S, **Piasecka A**, Song B, Dahms E, Sakakibara H, Gan X, Kułak K, Sawikowska A, Krajewski P, Tsiantis M, Garrido-Oter R, Fukushima K, Schulze-Lefert P, Laurent S, Bednarek P, Tsuda K.* Gene expression evolution in pattern-triggered immunity within *Arabidopsis thaliana* and across *Brassicaceae* species. *Plant Cell* 2021; 33, 1863-1887; doi:10.1093/plcell/koab073.;

IF_{2020}=11.277; $MNiSW_{2020}$=200; $MNiSW_{2024}$=200; C=21

My contribution to the creation of this publication consisted of: planning the experiments (together with the corresponding author), developing the research methodology, conducting the experiments, analyzing the data, interpreting the data (with the involvement of other co-authors).

H8. Czerniawski P, **Piasecka A**, Bednarek P*. Evolutionary changes in the glucosinolate biosynthetic capacity in species representing *Capsella, Camelina* and *Neslia* genera. *Phytochemistry* 2021; 181:112571; doi: 10.1016/j.phytochem.2020.112571;

IF_{2020}= 4.072; $MNiSW_{2020}$=100; $MNiSW_{2024}$=100; C=19

My contribution to the publication consisted of: analyzing and interpreting the obtained metabolomic data, writing a section of the first version of the manuscript on metabolomic

analyses, editing and improving the final version of the manuscript (with the help of other co-authors).

The name of the habilitant is in bold; * corresponding author; # authors who contributed equally to the work.

Table 1. Scientometric data on publications included in the habilitation achievement and all publications of the habilitant according to the Web of Science Core Collection database, as of April 12, 2024.

	Cycle (8)	**All publication with IF (27)**
IF	50.518	110.796
MNiSW	890	1667
$MNiSW_{2024}$	1030	2670
Number of citations (excluding self-citation)	176 (167)	1046 (960)
H-index	4	16

IF: Impact Factor for the year preceding the date of publication;

MNiSW: Points awarded by the Ministry of Science and Higher Education for the year preceding the date of publication;

$MNiSW_{2023}$: Points awarded by the Ministry of Science and Higher Education (according to the latest "List of scientific journals and peer-reviewed materials from international conferences" published on 17 Jul 2023).

Table 2. Total scientific achievements divided into periods before and after obtaining the doctoral degree.

Publication type		before obtaining the doctoral degree	after obtaining the doctoral degree	total
1.	Original publication with IF	3	24	26
2.	Abstracts	2	23	25
3.	Conference presentations	2	12	14
4.	Posters	2	23	25

Statements confirming the contribution of the Applicant in the creation of the above-mentioned papers are included in Appendix No. 9. Copies of the above publications are included in Appendix No. 8.

4.3. Discussion of the habilitation achievement

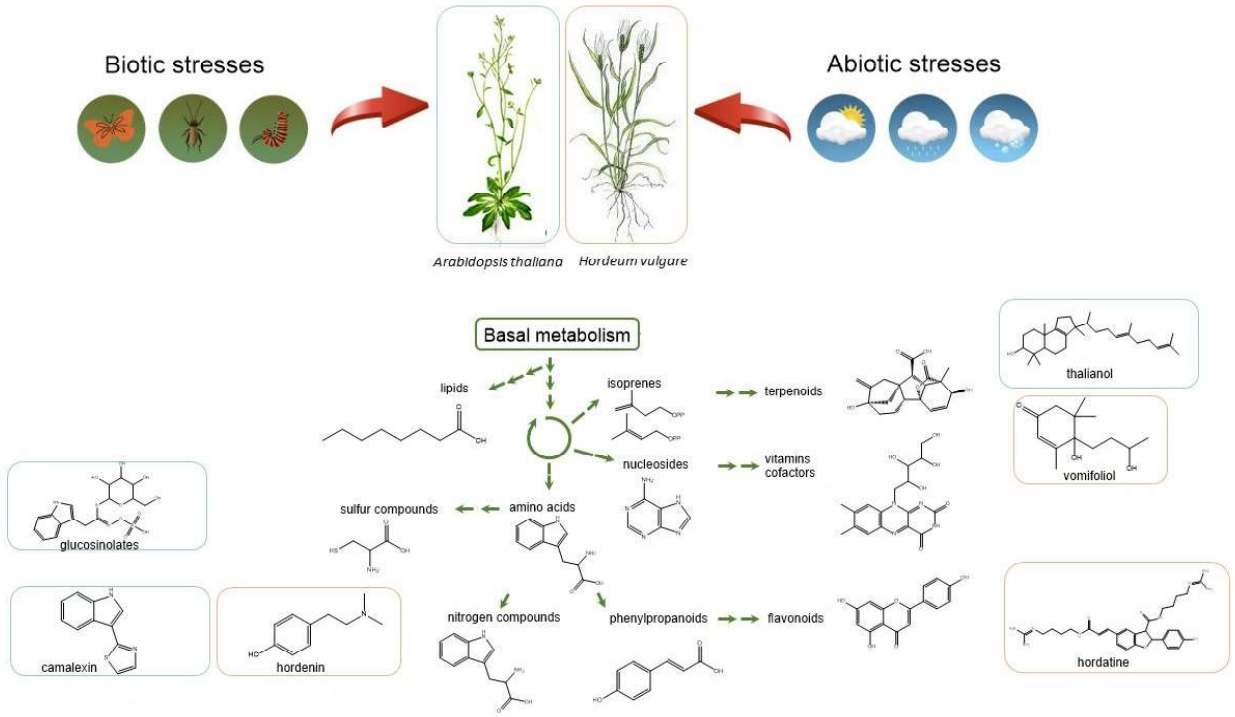
4.3.1. Introduction

Abiotic and biotic stresses have a detrimental impact on the fitness and productivity of crop plants. Climate changes mean that crops are constantly exposed to drastic environmental stresses leading to a forecast of lower global crop production. The tendency to increase the duration and severity of environmental stresses, such as drought or floods, will not only negatively affect the yield but also enhance the invasion of drought-adopted pathogens and herbivores (Liu and Liu, 2016). Therefore, it is necessary to introduce new varieties of crop plants that can withstand these unfavorable conditions.

Low-molecular-weight compounds are essential components of plant metabolism. Plants produce a large and diverse collection of such structures, which play a role in signaling pathways, regulation of many bioprocesses, and defense mechanisms against a wide range of biotic and abiotic stresses (Böttger et al., 2018). A significant group of metabolites regulates osmotic pressure and photosynthesis processes in abiotic stresses and also acts as antioxidants preventing the oxidation of cellular components (Szepesi, 2020)). They have long been considered as factors influencing plant interactions with other organisms, including symbiotic microorganisms (Wolinska et al., 2021), as well as pathogens and pests (Piasecka et al., 2015a). Metabolites produced by plants are extensively studied in the processes of plant responses to environmental stresses, as their elements, contributing to the limitation of the negative effects of stress on plants. Specialized metabolites have a wide spectrum of activity, and the accumulation of phenylpropanoids, flavonoids, stilbenoids, and fatty acids can be observed in many plants after pathogen infection (Bollina et al., 2010); and in abiotic stresses, such as drought (Piasecka et al., 2017) or excessive UV radiation (Ferreyra et al., 2021)). Therefore, it can be assumed that these structural groups constitute key elements of plant responses to external stimuli. Some groups of secondary metabolites, such as terpenoids, phenylpropanoids, and flavonoids, are widespread throughout the plant kingdom, suggesting similar, conserved biological functions of these compounds. On the other hand, elements of conserved biochemical pathways have undergone dynamic evolution, resulting in the occurrence of metabolites

specific to a limited group of plants, e.g., in taxonomically related genera or species, or even in a single species (Figure 1) (Wang et al., 2019). An example is group of metabolites specific to the *Brassicaceae* family belonged to tryptophan-derived and sulfur-containing structures like camalexin and glucosinolates. Next examples can be terpenoid thalianol produced only in *A. thaliana* a model plant for Brassiceae species (Field et al., 2011). Metabolites characteristic of the *Poaceae* family represented by barley (*Hordeum vulgare*) include the indole compound hordein and hordatins, which are conjugates of hydroxycinnamic acids with the polyamine agmatine.

Figure 1. Plant specialized metabolites involved in response to environmental stimuli are structurally diversified. All came from primary metabolism and shared first steps of biosynthetic pathways. Some of them are conserved among plant kingdom and others are species- or genera-specific. Metabolites characteristic to *Brassicaceae* represented by model plant *Arabidopsis thaliana* are marked with blue circles. Metabolites characteristic to *Poaceae* represented by *Hordeum vulgare* are marked



with red circles.

Understanding the molecular mechanisms underlying plant stress responses is extremely important for the development of new plant varieties with increased stress tolerance. In recent years, scientists have become increasingly interested in the possibility of using specialized metabolites as sources of biomarkers of plant tolerance to various stresses (Sakurai, 2022).

Metabolomics, which involves the comprehensive analysis of small molecules in biological systems, has emerged as a powerful tool for studying interactions between plants and

environment. When plant receptors are stimulated by stress signals, the expression of stress-responsive genes is activated, among others contributing to changes in the profile of secondary metabolites (Hong et al., 2016). Rapid screening analyzes help identify the phenotypic and molecular response of plants to abiotic and biotic stresses and search for stress-resistant individuals. Additionally, they help revealing the genetic and biochemical mechanisms underlying the plant plasticity for future genetic engineering of stress-resistant/tolerant plants. Thanks to metabolomics, it is possible to gain insight into metabolic pathways that are elements of plant responses to stresses and to identify potential biomarkers of stress tolerance (Hong et al., 2016). Metabolomics has become a valuable tool in various scientific fields, including agriculture, medicine, food and nutritional sciences, toxicology, functional genomics, and nutrigenomics.

Liquid chromatography (LC) or its combination with mass spectrometry (LC-MS) is most often used for metabolomics studies. Recent advances in mass spectrometric techniques have enabled high-throughput profiling of metabolites, facilitating the identification of key metabolites involved in plant stress responses. Factors contributing to the widespread use of mass spectrometric techniques in plant sciences include the ease of coupling with separation methods and the ability to use different physical phenomena for ionization and separation. Nuclear magnetic resonance (NMR) methods are an alternative approach to metabolome studies (Nagana Gowda and Raftery, 2021). Although they have lower sensitivity, they offer straightforward quantitative comparisons. Both NMR and MS-based methods can be used complementarily in plant sciences (Piasecka et al., 2015b).

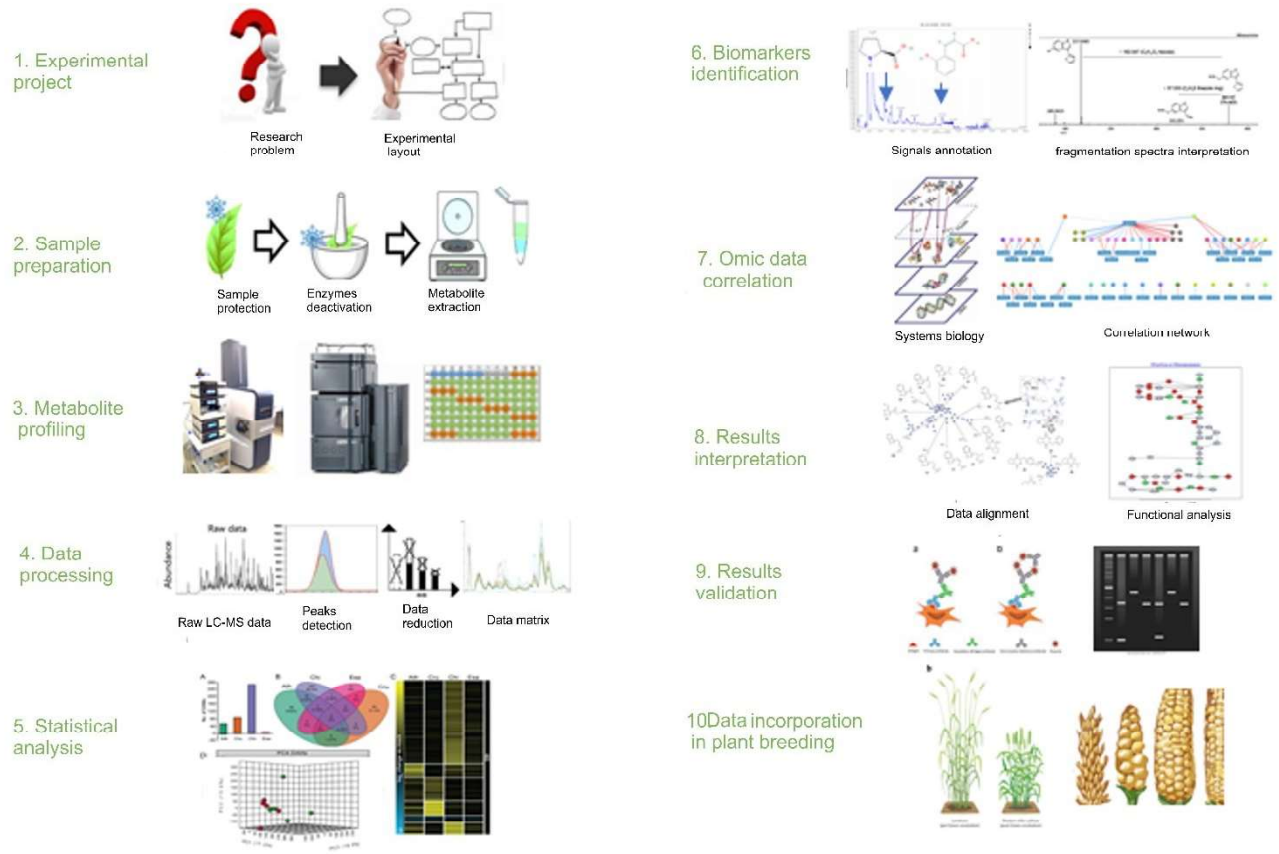
Identification of molecular markers through plant metabolomics requires a comprehensive approach that encompasses sample preparation, data acquisition, follow by data processing and curation, statistical analysis, structural identification of interesting signals, and results validation. (Figure 2) (Piasecka et al., 2019). Proper planning of experiments is crucial for driving research hypotheses. Collection and preservation of material should ensure the extraction of a wide spectrum of metabolites, from hydrophilic to hydrophobic, which are observed in plants. This is particularly important for analyzing specialized metabolites, such as glucosinolates, which require laborious sample preparation and can be complex to analyze. Using different solid phases and solvents in sample preparation can reduce the number of natural products present in the analyzed samples, resulting in increased sensitivity and dynamic range in MS instruments. With thousands of possible specialized metabolites in a single sample, selecting appropriate MS approaches and optimizing parameters for the ion source and detector

is just the first step in the lengthy process of obtaining meaningful data in MS analysis. Metabolomic analysis includes both untargeted and targeted approaches. The untargeted approach is useful for monitoring qualitative and quantitative changes in sample composition and enable the hypotheses formulation. This analytical approach has proven to complement other omics techniques, identifying metabolomic markers that can be used to improve the nutritional composition of maize (Venkatesh et al., 2016), or rice (Silva et al., 2017), or traits that contribute to improved yield under environmental stresses in maize (Obata et al., 2015), rice (Degenkolbe et al., 2013) or sorghum (Borrell et al., 2014). A targeted (targeted) approach is used to study a specific known group of chemical compounds in samples. Using internal standards, the analysis can be performed in a quantitative manner. This method allows monitoring of a wide range of enzymes, their kinetics, as well as components of metabolic pathways. When targeted metabolomics is used, sample preparation can be optimized, reducing the impact of background elements created in the detector. In addition, although clearly defined metabolites are analyzed, new compounds can also be identified in the context of specific physiological states.

The sensitivity of MS devices has led to a significant increase in the number of detected signals during each chromatographic run of studied samples, resulting in very large data files. It is therefore necessary to reduce the dimensionality of the data using appropriate mathematical methods. For this purpose, advanced tools have been developed to process raw LC-MS data into a matrix that can then be subjected to statistical analysis. Data processing and alignment tools are essential for creating a data matrix suitable for statistical analysis. One of the biggest challenges in plant metabolomics is extracting useful information about specialized metabolites from the vast amounts of data produced by modern LC-MS systems. To accomplish this, data curation followed by advanced statistical methods is necessary to compare all signals across all

samples and quickly identify relevant signals. The interpretation of data relies on the use of existing programs for identification and statistical analysis.

Figure 2. Mass spectrometric techniques in functional metabolomics flowchart—a useful tool for



metabolome profiling and discovery metabolomic biomarkers of plant abiotic and biotic stress. Schematic presentation of the individual stages of research on a comprehensive analysis of plant metabolites related to environmental stresses using modern plant metabolomics approaches.

4.3.2. Motivation and purpose of research

The scientific achievement presented here comprises a cycle of eight publications, including seven experimental papers and one review. These works resulted from scientific grants where I participated as an Investigator or a Principal Investigator. I am the first author in five of these works and the corresponding author in one. All papers have been published in journals listed in the Journal Citation Reports (JCR) database, accumulating a total impact factor (IF) of 49.667 and 167 citations (as of 12.04.2024, according to the Web of Science Core Collection). This series of work focuses on identifying metabolomic components associated

with crop plant responses to environmental stresses using mass spectrometry and liquid chromatography.

The research presented in papers **H1-H3** was part of a multidisciplinary project aimed at exploring the genetic, ecophysiological, morphological, anatomical, biochemical, and biophysical aspects of cereal tolerance to water scarcity. It was conducted under the Operational Program Innovative Economy "Biotechnological tools for obtaining cereal varieties with increased resistance to drought, POLAPGEN-BD" No. WND-POIG.01.03.01-00-101/08, POIG. My research was supervised by Prof. Piotr Kachlicki in the Metabolomics Team at the Institute of Plant Genetics, Polish Academy of Sciences. The project started with the hypothesis that barley harbors a rich and unexplored array of secondary metabolites, some of which are implicated in the plant's response to water deficiency and acclimation processes. Additionally, the content of these secondary metabolites varies among varieties from different parts of the world. Understanding these response mechanisms to environmental stress is crucial for developing new crop plant varieties tolerant or resistant to such stresses.

The primary goal of research in **H1-H3** was to apply metabolomic and chemometric approaches to study barley's molecular phenotype and search for metabolomic biomarkers of drought resistance in crop plants. As part of the POLAPGEN-BD project, I published four research papers, three as the first author. One of the publications, "Piasecka et al. 2015. Combined mass spectrometric and chromatographic methods for in-depth analysis of phenolic secondary metabolites in barley leaves. *Journal of Mass Spectrometry* 2015; 50 (3): 513-532," was closely related to my PhD thesis on metabolite identification in nine barley genotypes and the variability in phenolic metabolites among genotypes from different climatic conditions. The use of complementary LC-MS^n (mass spectrometry with multistep fragmentation), LC-HRMS (high-resolution mass spectrometry enabling chemical formula prediction), and NMR techniques for structure confirmation allowed for the characterization of 152 phenolic secondary metabolites. These identified metabolites were further analyzed for changes in the barley metabolome under drought influence using liquid chromatography with photodiode array detector (UPLC-PDA) methods and correlated with genomic data, as described in **H1**. In **H1**, an attempt was made to understand the molecular basis of barley's response to drought stress by mapping loci of metabolomic quantitative traits controlling physiological and biochemical traits related to water shortage response. This was enabled by constructing a high-resolution, consensus genetic map of SSR and SNP markers for three RIL populations derived from crosses between European and Syrian spring barley varieties.

In my **H2** publication, I proposed an innovative method combining metabolomics with chemometrics to describe differences in metabolome mobilization during drought between plant vegetative and generative phases and their correlation with phenotypical consequences. Observations of plant yield and biomass provided insights into moisture deficit's developmentally related long-term consequences on plant fitness. A significant challenge in the project was implementing and optimizing the metabolite profiling method using UPLC-PDA methods.

The undeniable challenge of the project was the implementation and optimization of the metabolite profiling method using the UPLC-PDA method. In **H3**, I participated in work that led to creating innovative methods for processing such data, with a special emphasis on separating overlapping peaks which co-eluted during chromatographic separation. **H3** also bridged the POLAPGEN-BD project with my subsequent project, using some data from the latter for method evaluation.

Following the POLAPGEN-BD grant, I received an NCN SONATA research project funded by the Polish National Science Center (NCN). My research was conducted at the Institute of Plant Genetics, Polish Academy of Sciences, in the Metabolomics Team (under Prof. Piotr Kachlicki supervision) and simultaneously at the Institute of Bioorganic Chemistry, Polish Academy of Sciences, in the Department of Functional Plant Metabolomics (under Prof. Paweł Bednarek supervision). This project was established with the intention of developing my competence in metabolomic techniques and expanding my knowledge of the contribution of the plant metabolome to biotic stresses. The project's goal was to identify metabolomic biomarkers of tolerance to the mycotoxinogenic pathogen *Fusarium* in *Poaceae* family plants (https://projekty.ncn.gov.pl/index.php?projekt_id=290019). This pathogen significantly reduces cereal crop yield worldwide and produces mycotoxins in grains. The project focused on important crops like wheat (*Triticum aestivum*) and barley, and model plant purple false brome (*Brachypodium distachyon*) genotypes with varying susceptibility to *Fusarium* infection. The search for immunity-related metabolites in the grass family holds immense potential for plant breeding and metabolic engineering in agriculture, offering a means to intensify control over existing yield losses (Sakurai, 2022).

The research tasks were structured around mass spectrometry techniques, including LC-MS/MS (tandem mass spectrometry, which enables the acquisition of fragmentation spectra MS2) and LC-MS^n (mass spectrometry with multi-step fragmentation from MS1 to MS5). A significant challenge during the project's implementation was the development and

optimization of methodologies for processing LC-MS plant metabolomics data and the implementation of efficient statistical methods for a multifactorial experiment to derive biological insights. To master the methodology related to the analysis and processing of non-targeted LC-MS analyses, I completed the "Metabolomics Data Processing and Data Analysis" online course, organized by the University of Birmingham, and participated in the "Metabolomic Bio & Data" workshop in Vorau, Austria. Additionally, I undertook a short-term internship at the Georg-August-University of Göttingen, Albrecht-von-Haller-Institute for Plant Sciences, Department of Plant Biochemistry, to train in metabolomics data processing.

This acquired knowledge was encapsulated in the **H4** review. The focus of this work was on developing a flowchart for metabolomic experiments to efficiently extract metabolomic markers. This methodology included defining Differentially Accumulated Metabolites (DAMs), analogous to the definition used in transcriptomic analyses, and Differentially Expressed Genes (DEGs), which assisted in understanding the specificity of the compounds searched and facilitated the correlation of metabolomics data with other omics results. The established flowchart and methodologies for plant metabolomics were then applied to conduct profiling experiments, identifying metabolites specific to *Poaceae* genotypes with varying susceptibility to *Fusarium* infection, as detailed in the **H5** publication. The study incorporated metabolomics profiling with functional analysis of biochemical pathways and correlation analysis, distinguishing between the conserved elements of stress response and the unique elements of specific genotypes.

I also completed a short-term scientific internship at the Institute of Natural Fibers and Medicinal Plants in Poznań, focusing on measuring plants' antioxidant potential. This training enabled me to measure physiological parameters such as antioxidation status and infection progression level, aiding in the selection of plant materials that most distinctly exhibited susceptibility to the pathogen. My expertise in plant biochemistry and physiology was successfully combined in **H5** with modern mass spectrometry techniques and computational methods, contributing to my ongoing professional development.

In **H6**, an in-depth description of the metabolome of *B. distachyon*, proposed as a model system for the functional genomics of grasses, was published. Until this point, the metabolome of this plant was relatively unexplored. A comprehensive description of metabolites, based on mass spectra fragmentation and database annotation, was enhanced with a functional analysis of the metabolic pathways in the *B. distachyon* metabolome. This analysis was pivotal in demonstrating the conservation of function between *B. distachyon* and cereals in terms of

disease defense at the metabolomic level, concluding the series of metabolomic biomarkers of environmental stimuli in *Poaceae* plants. **H6** completes a series of metabolomic analyses related to mechanisms of environmental stimulation in plants of the *Poaceae* family.

My primary motivation in research is to acquire extensive knowledge about the role of plant metabolites in stress response. Participating in an international project investigating the immune system of cruciferous plants provided an opportunity to explore wider range of phytochemical structure in other plant families than *Poaceae*. **H7** describes the study of the evolution of pattern-triggered immunity (PTI) responses, analyzing transcriptomic and metabolomic changes in *A. thaliana* and other *Brassicaceae* species exposed to microbe-associated molecular patterns (MAMPs). The objective was to identify the genes and metabolites that commonly and specifically respond to MAMP which was bacterial flagellin epitope (flg22). The observed differences in the metabolomic profiles among closely related species described in **H7** were further investigated in **H8**. It involved a comparative analysis of glucosinolate distribution and biosynthesis in different organs of species closely related to *A. thaliana* within the *Camelineae* tribe (*Brassicaceae* family), complemented by identifying orthologs of glucosinolate biosynthetic genes in their published genomes. Both genetic and metabolomic investigations provided insights into the evolutionary changes in the glucosinolate biosynthesis pathway within the *Camelineae* tribe. **H8** represents an interdisciplinary study showcasing a range of modern analytical approaches and techniques across biochemistry, molecular biology, computational biology and bioinformatics, culminating the research cycle.

The main goal of my research was to identify specific plant metabolomic response elements to environmental stress using chromatographic and mass spectrometry techniques.

Certainly, the **specific objectives** from the previously described research can be outlined as follows:

1. Identification of metabolomic components in crop plant responses to environmental stresses:
   - Utilizing mass spectrometry techniques to identify key metabolomic components that play a role in crop plants' response to environmental stresses, particularly focusing on the grass family.
   - Correlation of the metabolomic profile with the genetic background of plants subjected to environmental stress.
2. Study of barley's molecular phenotype and drought resistance:

- Applying metabolomic and chemometric approaches to investigate the molecular phenotype of barley.

- Searching for molecular biomarkers of drought resistance in barley and other crop plants.

3. Comprehensive analysis of phenolic secondary metabolites in barley and model plant *B. distachyon:*

- Characterizing a wide range of phenolic secondary metabolites in different genotypes and organs.

- Understanding the variability of these metabolites among genotypes from various climatic conditions.

4. Development and optimization of methodologies for plant metabolomics:

- Developing and optimizing the processing methodologies for LC-PDA plant metabolomics data.

- Developing and optimizing the processing methodologies for LC-MS plant metabolomics data.

- Implementing efficient statistical methods for multifactorial experiments to extract biological insights.

- Mastering the methodology related to the analysis and processing of non-targeted LC-MS analyses.

- Gaining training in metabolomics data processing to enhance the quality and efficiency of data analysis.

5. Identification of metabolomic biomarkers for *Fusarium* tolerance in *Poaceae* family:

- Identifying metabolomic biomarkers related to tolerance against the mycotoxinogenic pathogen *Fusarium* in the *Poaceae* family.

- Demonstrating the conservation of function in disease defense at the metabolomic level between *B. distachyon* and cereals.

- Focusing on economically significant crops like wheat and barley, and model plants like purple false brome (*B. distachyon*) in a context of plant immunity variability.

6. Investigation of Pattern-Triggered Immunity (PTI) responses in *Arabidopsis thaliana* and other *Brassicaceae* species:

- Studying the transcriptomic and metabolomic changes in A. thaliana and other *Brassicaceae* species upon exposure to MAMPs.

- Identifying genes and metabolites that respond to MAMP flg22 and understanding the evolution of PTI responses in these species.

- Correlation of the metabolomic profile with transcriptomic data in an evolutionary context of plant immunity variability.

7. Comparative analysis of glucosinolate biosynthesis in the *Camelineae* tribe:

- Conducting a comparative analysis of glucosinolate distribution and biosynthesis in different organs of selected species within the *Camelineae* tribe.

- Identifying orthologs of glucosinolate biosynthetic genes and understanding evolutionary changes in the biosynthesis pathway.

4.3.3. Discussion of the results

Identification of metabolomic biomarkers of barley response to drought (H1-H2)

Moisture deficit is a significant stress factor that limits plant growth and productivity globally. Plant resistance to moisture deficit is affected by various factors, including soil conditions, climate, farming practices, concurrent stresses, and the plant's developmental stage. Understanding these factors is essential for devising effective strategies to manage moisture deficit. The complexity of plant resistance to drought, encompassing genetic, physiological, and morphological adaptations influenced by the drought's severity and the plant's developmental stage, necessitates a systematic approach for resolution.

The **H1** study aimed to identify metabolomic biomarkers that significantly change under moisture deficit conditions and to explore the impact of genetic background on these changes using the mQTL approach. This was to enhance our understanding of barley's response mechanisms to drought stress. Additionally, the study sought to establish statistical gene-to-metabolite networks focusing on sub-groups of secondary metabolites. The methodology adopted facilitated the identification of 98 drought-related traits. Analysis unveiled notable alterations in the levels of phenolic and terpenoid compounds, with hydroxycinnamic acid derivatives prevailing under extended drought conditions, whereas simple phenylpropanoid derivatives were predominantly up-regulated in the initial phase of drought stress. Phenolic compounds, comprises a subgroup of secondary metabolites, play a crucial role in responding to and tolerating moisture deficit, serving as protective agents against UV radiation. Metabolites featuring methoxyl groups, particularly ferulic acid and its derivatives, were identified as specialized drought-responsive elements. Phenolic compounds, acting as substantial non-

enzymatic antioxidants, play a critical role in plants' response and tolerance to moisture deficit by dissipating energy and preventing photoinhibitory damage to the photosynthetic apparatus (Hura et al., 2008). The data obtained support the conclusions of a paper (Schmitz-Hoerner and Weissenböck, 2003), which demonstrated the role of phenolic compounds in protecting DNA from damage caused by excessive UV radiation. Methylation processes of hydroxycinnamic acids increase the range of absorption of UV wavelengths, and the combination of these acyls with flavonoids significantly enhances the intensity of absorption (Stochmal and Oleszek, 2007). At the same time, processes of blocking the hydroxyl groups of flavonoids by glycosides were observed, which reduces their reactivity and allows the accumulation of these metabolites in the vacuole. In addition, studies have shown that blumenol metabolites and polyamine derivatives of hordatines hitherto linked to plant immune response, may have unknown functions in barley's response to drought.

Moreover, the **H1** study pinpointed genomic regions and candidate genes for regulating metabolite levels or groups of metabolites under drought conditions. Most of the identified mQTLs were associated with flavonoid derivatives early in the drought phase, mainly flavones glycosylated at the 2″-*O*- position, which could be crucial for their transport to vacuoles for storage. Interestingly, the study found almost no direct associations between metabolites and their biosynthetic genes but identified numerous genes associated with defense response processes connected to the mQTLs.

The **H1** findings suggest that metabolites are essential as antioxidants, gene expression regulators, and protein function modulators in barley plants under moisture deficit stress. Identifying metabolomic quantitative trait loci within genomic regions associated with stress response could aid in breeding stress-tolerant crops.

Conversely, **H2** explored phenomena related to plant acclimation to water deficiency and drought consequences, finding correlations between the accumulation of specific metabolites, like sinapic acid derivatives and flavonoids, and particular phenotypic features such as lateral stem and straw yield. Drought-induced metabolomic reprogramming was mainly attributed to the increased accumulation of methyl group-containing compounds. Acclimation to water deficiency, a non-inheritable adaptation, alters plant structures and functions, with drought leading to an excessive accumulation of secondary metabolites, impacting physiological processes and biomass and yield production. The correlation between sinapic acid accumulation and phenotypic features underscores the importance of these compounds in cell

wall fortification. Flavonoids, especially those with mono-hydroxyl B-ring structures, were linked to auxin transport and essential for biomass growth under water-deficit conditions. The study also highlighted the significance of metabolomic reprogramming during drought, primarily due to the increased accumulation of methyl group-containing compounds and a variety of glycosidic flavonoids. LC-MS^n methods facilitated the detection of new drought-related metabolites, emphasizing the acylation of flavonoids, sometimes even with two hydroxycinnamic acid moieties, as a vital drought response. This acylation significantly increases UV-B radiation absorption, potentially serving as a protection strategy against UV radiation-induced damage upon photosystem failure in barley due to drought. However, it seems that the shift of the UV absorption maximum towards longer wavelengths is the most important effect of the methylation occurring in drought, as previously suggested (Stochmal and Oleszek, 2007). It is noteworthy that in wheat, drought transition results in an increase in UV-B tolerance, and vice versa, indicating cross-talk between drought and UV-B tolerance mechanisms (Veselá et al., 2022).

Optimization of metabolic data processing flowchart from plant material (H3 and H4)

In recent years, metabolomics has emerged as a critical field of research in the development of stress-tolerant crops, with a particular focus on model and agricultural species. This discipline has elucidated the biochemical and physiological underpinnings dictating plant resilience or vulnerability to diverse stressors. Metabolite profiling, employing liquid chromatography coupled with PDA) and fluorescence (FLR) detection—for analytes such as carotenoids, chlorophylls, phenolics, flavonoids, and betalains—offers a rapid and precise methodology for the quantitative analysis of a broad spectrum of metabolites. HPLC-PDA detection is frequently utilized for mycotoxin quantification in foodstuffs and metabolite profiling. Nevertheless, when liquid chromatography is applied without tandem mass spectrometry, co-elution of compounds can complicate both qualitative and quantitative analysis due to peak overlap, a prevalent issue in chromatographic methodologies. The **H3** publication introduces two novel computational strategies for the resolution of overlapping peaks in extensive chromatographic data sets, which have undergone empirical validation. The initial method leverages clustering algorithms, whereas the second employs functional principal component analysis (FPCA). Both techniques involve data normalization, baseline correction, alignment of retention times, and peak detection. The applicability of these methods for

disentangling overlapping peaks is supported by findings from both simulated and empirical studies examining metabolomic alterations in barley foliage subjected to drought stress. Notably, FPCA offers the added benefit of evaluating the variance of individual compounds within identical peaks across different chromatograms, as evidenced by a selection of large-scale data sets derived from ultra-performance liquid chromatography with photodiode array detection (UPLC-PDA) analyses of barley's response to environmental stimuli.

Furthermore, advancements in the sensitivity and selectivity of LC-MS technology have facilitated the generation of voluminous data sets from multi-sample experiments within the realm of metabolomics. This progression necessitates the development of sophisticated data processing tools to manage the augmented complexity of such data, thereby enhancing the accuracy and reliability of metabolomic investigations. Nonetheless, the intricate and multistep nature of LC-MS data processing can inadvertently introduce errors, potentially leading to erroneous conclusions. To mitigate this risk, it is imperative for researchers to possess a thorough understanding of the data processing workflow, including the requisite transformations and adjustments applied to raw data before the selection of analytical parameters.

My comprehensive preparation for engaging with plant metabolomics via LC-MS summarized in the **H4** review, focusing on the plant's response to environmental stressors. To my knowledge, this publication represents the inaugural overview offering an exhaustive delineation of the sequential data processing algorithms employed in this field. It underscores the importance of method validation and the integration of metabolomic data with other systems biology levels to attain a holistic comprehension of the molecular dynamics governing plant metabolism under stress conditions. The review also highlights the critical role of identifying molecular biomarkers to facilitate the breeding of crops with enhanced stress resistance and yield. It outlines various methodologies for the discovery of stress-responsive biomarkers, which could prove instrumental in marker-assisted selection. Additionally, the review contrasts analytical techniques based on gas chromatography (GC) and nuclear magnetic resonance (NMR) with LC-MS approaches, noting the former's reduced sensitivity but straightforward qualitative analytical capabilities.

Identification of metabolomic biomarkers of Poaceae plants response to Fusarium infection (H5)

The devastating impact of *Fusarium* spp., especially *Fusarium culmorum*, which causes *Fusarium* head blight (FHB) on cereal crops worldwide, results in yield reduction and mycotoxin production, such as zearalenone (ZEN) and deoxynivalenol (DON). Although some progress has been made in understanding the defense mechanisms against *Fusarium* infection in certain grass species, i.e., describing QTLs associated with FHB resistance in barley (Huang et al., 2018), there is still limited knowledge of the molecular response to FHB common to many grasses. Various defensive metabolites are derived via the phenylpropanoid/shikimic acid pathway in *Poaceae* plants. The accumulation of hydroxycinnamic acid amides and phenolic acid glycosides in wheat's response to FHB was correlated with cell wall thickening and the inhibition of pathogen growth. The key enzymes of phenylpropanoid biosynthesis were up-regulated in response to *Fusarium* and DON production. Recent attention has also been paid to the antifungal properties of polyamines, with putrescine, spermidine, and spermine noted as rapidly induced in FHB and contributing to the induction of DON biosynthesis by *F. graminearum* (Wojtasik et al., 2015). Polyamines acylated with hydroxycinnamic acids accumulate during pathogenesis in many grass species.

Multidisciplinary studies described in **H5** showed differences in molecular and physiological changes in the grains of barley and wheat genotypes with diversified resistance to FHB, as well as in the model grass *B. distachyon* (Bd). Firstly, quantitative measurements of *F. culmorum* DNA and its toxins made it possible to visualize the progression of infection in different genotypes of *Poaceae* plants and to distinguish genotypes with increased and decreased susceptibility to the pathogen. Susceptible genotypes of barley and wheat had a higher level of fungal biomass compared to resistant genotypes, while *B. distachyon* had intermediate resistance to *F. culmorum*. The study also looked at the accumulation of mycotoxins, including trichothecene mycotoxins and estrogenic zearalenone, which were positively correlated with pathogen biomass in plants. The accumulation of mycotoxins was significant in all genotypes and could affect the physiological and molecular changes in plants after *F. culmorum* inoculation. Additionally, the observed prevalence of zearalenone can affect the fungal aggressiveness in plant spikes. The study suggests that understanding the structural dependence of mycotoxins on plant immunity could improve our knowledge of plant-pathogen interactions in FHB.

Antioxidant capacity and low-molecular antioxidants play crucial roles in ROS detoxification and maintaining photosynthetic balance. *F. culmorum*, a hemibiotrophic pathogen, benefits from the dead tissues damaged by ROS. In resistant genotypes of barley and

wheat, the antioxidant capacity remained constant even during advanced *F. culmorum* infection, while the accumulation of secondary metabolites increased. The observation allowed the suggestion that resistant genotypes exhibited high antioxidative system efficiency, which restrained the progress of pathogenesis. The compensation for antioxidative potential under infection was observed by increasing the low-molecular antioxidant content in all studied genotypes. The physiological response to *F. culmorum* of *B. distachyon* was found to be close to that of resistant barley and wheat genotypes; however, metabolomic changes were strongly diversified.

The main aim of **H5** was to identify similarities and differences in the immune responses to *F. culmorum* among different grass species and genotypes. *F. culmorum* infection caused massive metabolomic reprogramming, but with a high level of conservativeness among *Poaceae* plants. The study also found enrichment in specific metabolic pathways related to central carbon metabolism and evolutionarily conserved phenylpropanoids, flavonoids, and porphyrin biosynthesis. The disturbance of sugar storage in seeds and germination processes and sugar metabolism were also commonly observed. The highest changes were observed for phenylpropanoic structures such as hydroxycinnamic acids, chalcones, flavanes, flavanols, and anthocyanidins, which are related to ROS scavenging or inhibition of lipid peroxidation. Finally, the study highlights the triggering of an efficient ROS scavenging pathway, "Ascorbate and Aldarate metabolism", engaged in a conserved immune response in *Poaceae* plants. It has been noted previously that the accumulation of hydroxycinnamic acid amides and phenolic acid glycosides in response to FHB is associated with cell wall thickening (Gunnaiah et al., 2012). In wheat, an FHB-resistant genotype showed increased accumulation of p-coumaric acid in spikelet tissues compared to a genotype susceptible to *F. culmorum* infection (SIRANIDOU et al., 2002). Phenylalanine ammoniacyanase (PAL), an enzyme involved in the biosynthesis of the phenylpropanoid pathway, plays a key role in wheat resistance to *F. graminearum* and the action of the mycotoxin deoxynivalenol (DON). Increased transcription of PAL in infected wheat ear tissue has been correlated with resistance to FHB (Steiner et al., 2009). Other key enzymes in phenylpropanoid biosynthesis, such as cinnamic alcohol dehydrogenase, caffeoyl-CoA *O*-methyltransferase, flavonoid *O*-methyltransferase and peroxidase, are also induced in response to *F. graminearum* and DON infection (Pasquet et al., 2016).

Resistance-related metabolites belong to different structural classes, including nitrogen-containing metabolites from "Caffeine metabolism" and amino acids such as proline, alanine, and serine. Sulfur-containing dicarboxylic acids and related compounds from "Cysteine and

Methionine metabolism" were also identified as resistance-related. Methionine and 12-hydroxyjasmonic acids were found to be related to susceptibility. Benzoxazinoids accumulation was observed to be correlated with resistance to FHB in wheat. We focused on the interaction between plants and the pathogen *F. culmorum*, and examined the production of antioxidants and phytoalexins, as well as their correlation with sugar management mechanisms and photosynthesis. The results incorporated the complex interplay between plant metabolites and pathogenic microbes, and the importance of conserved and genotype-specific elements in plant immunity. The article concludes that metabolomic studies have the potential to identify biomarkers of resistance to FHB and aid in the development of crop species with improved resistance.

Brachypodium distachyon metabolome characterisation emphases genotypes and organ specificity (H6)

The purple false brome (*B. distachyon*) is a plant model for genomic studies that is closely related to wheat and barley (Draper et al., 2001). It has a small genome and a rapid life cycle, making it an ideal subject for research. Since its genome was sequenced, *B. distachyon* has been extensively studied and has been utilized as an excellent model system for the study of cereal crop-pathogen interactions. Although it has been shown that *Fusarium* spp. infects *B. distachyon* similarly to the way it infects wheat (Peraldi et al., 2014), the specialized metabolites produced by *B. distachyon* remain largely unknown. Therefore, in the **H6** study, targeted and untargeted mass spectrometry techniques were used to identify specialized metabolites in *B. distachyon* accessions Bd21 and Bd3-1, which differ in their morphology, development, and stress tolerance.

The comparative analysis of spikes, leaves, and roots revealed metabolic pathways that significantly differentiated the analyzed organs and genotypes. A total of 93 specialized metabolites were tentatively identified based on two complementary LC-MS systems: a high-resolution LC-MS/MS orbitrap mass spectrometry system and an LC-MS^n ion trap mass spectrometry system. MS^n spectra were used to identify complex metabolites such as flavonoid glycoconjugates, while high-resolution MS/MS analysis was used to confirm the tentative structures predicted by the MS^n analysis. This unique combination of two LC-MS systems allowed for the description of structures specific to *B. distachyon* that cannot be annotated with automated bioinformatics approaches. These metabolites were categorized at levels 1-3 according to the Metabolomic Standards Initiative (Sumner et al., 2007).

Detailed inspection of mass spectra obtained during MS/MS and MS^n analyses of *B. distachyon* organs, combined with database and literature searches, focused on phenylpropanoids and flavonoid derivatives. Phenolic and hydroxycinnamic acids and their derivatives were found in high abundance in all the studied organs. *p*-coumaric, caffeic, and ferulic acids have been identified in conjugation with (methyl)quinic acids, sugar acids, or polyamines. Additionally, caffeic and ferulic acids have been observed as free molecules and identified by comparison to their standards.

The most abundant signals detected in all studied organs corresponded to sugar acid derivatives, particularly threonic acid conjugated to hydroxycinnamic acids. Additionally, 30 metabolites with the highest diversification among the studied genotypes and tissues were selected and visualized. The annotated compounds included derivatives of hydroxycinnamic acids, flavones, monounsaturated fatty acids, phosphate-containing, and sulfur-containing metabolites. Putative derivatives of hydroxycinnamic acids were highly represented, with conjugates of hydroxycitrate being particularly noteworthy. Flavonoids, such as putative derivatives of the flavone apigenin and proanthocyanidin B, also showed differentiation between lines. Interestingly, cyanidin 3-*O*-glucoside (chrysantemin) was already reported as a differentially accumulating metabolite in *B. distachyon* spikes previously (Onda et al., 2019). Finally, differences in phosphate management between the two lines were suggested based on the variable accumulation patterns of phosphoglycerolipids in all organs and lines.

Finally, methylthio-pentylmalic acid, a sulphur-containing metabolite from the 2-oxocarboxylic acid pathway, was identified as one of the most differentiated metabolites, with its dominant abundance in Bd3-1, particularly in roots. The **H6** study demonstrates that combined targeted and untargeted metabolomics can provide comprehensive knowledge about significant elements of the plant metabolome.

In the **H6** article, efforts were made to understand the metabolic differences between two *B. distachyon* lines, Bd21 and Bd3-1. The lowest differences were observed in roots, and the highest in spikes. The main differentiating compounds belonged to gibberellins, known as important factors for root elongation in monocots. Histidine and vitamin B6 metabolism had a shared effect on leaves and spikes, while the cysteine and methionine metabolism pathway specifically differed between the spikes of both lines, indicating possible differences in sulphate assimilation.

Identification of metabolomic biomarkers of the response of Brassicaceae plants in a pattern-induced immune response in an evolutionary context (H7 and H8)

In the H7 plant pattern-triggered immunity (PTI) was studied in evolutionary context. Despite the significance of PTI for plant adaptation to the environment, our understanding of PTI evolution remains limited. A comparative transcriptomic and metabolomic approach was used to address the evolution of PTI responses among different species of *Brassicaceae* plants. The international researcher group focused on a set of genes that commonly respond to the microbe-associated molecular pattern (MAMP) flg22 and found that variation in flg22-triggered transcriptome responses across *Brassicaceae* species was incongruent with their phylogeny. However, expression changes were strongly conserved within *A. thaliana* accessions. The study also found that salicylic acid (SA) signaling was responsible for sustained transcriptional reprogramming in *A. thaliana*. However, SA accumulation alone does not explain the distinct temporal dynamics of transcriptional reprogramming in these *Brassicaceae* plants. The study revealed species-specific gene expression patterns in the tested plants, and these genes may be involved in a collection of biological processes. Some genes showing species-specific expression patterns were associated with GO terms connected to secondary metabolism, prompting further investigation into the metabolite profiles of *Brassicaceae* plants.

I have discovered that the majority of flg22-induced changes in metabolite accumulation were specific to each species, indicating a significant diversification of the native metabolome and its reprogramming in response to flg22. These results suggest that flg22 treatment causes species-specific changes in the metabolome of *Brassicaceae* plants, emphasizing the diversity of metabolite responses to this MAMP. This interesting phenomenon was further expanded and complemented in the study described in **H8**, which investigated evolutionary changes in the biosynthetic pathway of glucosinolate production in different organs of members of the *Camelineae* tribe, including *A. thaliana, Capsella, Camelina,* and *Neslia* species. Glucosinolates are specialized metabolites that function in plant defense and contain sulfur and nitrogen. They consist of a β-D-thioglucose moiety connected to a sulfonated aldoxime with a side chain which properties are determined by the precursor amino acid. Glucosinolates can be classified into three groups: aliphatic, benzyl, and indolic derivatives. In **H8**, glucosinolate biosynthetic genes and evolutionary changes in their transcription and protein sequences were identified. We used LC-MS/MS to analyze extracts from various parts of the plants, including leaves, roots, inflorescences, and siliques, and identified 30 different glucosinolates, with *A. thaliana* producing the highest number of structures. All species were able to produce aliphatic

and indolic glucosinolates, but benzyl glucosinolates were only detected in *A. thaliana*. We concluded that the investigated species had a reduced structural diversity of glucosinolates compared to *A. thaliana*. The results of **H8** indicate that glucosinolates are highly abundant in siliques and roots but are hardly produced in the leaves of studied plants. The results suggest that the regulation of glucosinolate biosynthesis in studied species is different from that in *A. thaliana* and is likely related to their unique glucosinolate accumulation patterns in different organs. Additionally, there is reduced structural diversity of methionine-derived aliphatic glucosinolates with elevated accumulation of rare long-chain aliphatic glucosinolates, which is correlated with evolutionary changes in genes encoding methylthioalkylmalate synthases. The conservation of long chain aliphatic glucosinolates in the *Camelineae* tribe suggests an adaptive advantage due to their enhanced biological activity, but the impact of the chemical nature, including the length, of the side chain on ITC toxicity towards herbivores or pathogens is not fully understood. Finally, the biosynthetic pathway for tryptophan-derived indolic glucosinolates is likely on its evolutionary route to be shut off in the investigated species.

4.3.4. Summary and perspectives

My scientific achievement has had several important effects, including:

• Developing and optimizing a methodology for comparative metabolomics assisted by statistical approaches to search for plant biomarkers of drought resistance.

• Demonstrating that integrating metabolomics with genomics or phenotypic data offers a novel perspective on searching for biomarkers of drought resistance from a systems biology perspective.

• Developing and optimizing a methodology for conducting a comprehensive metabolomic analysis of plant immunity-related elements.

• Demonstrating the effectiveness of using liquid chromatography-mass spectrometry-based metabolomics for searching for plant biomarkers of *Fusarium* resistance in the *Poaceae* family.

• Comparing and identifying resistance-dependent metabolic and signaling processes in plants with high and low resistance to *Fusarium* infection in the *Poaceae* family.

• Describing the metabolome of different organs and genotypes of the model *Poaceae* plant, *B. distachyon*.

• Highlighting the importance of metabolomics in studying evolution in plant immunity.

• Demonstrating the dynamic changes in the metabolomic composition during the evolution of *Camelineae* plants.

In conclusion, my research has advanced the field of metabolomics and its application in plant science, particularly in the study of plant resistance to environmental stresses and diseases. Future studies could build on these findings to develop practical applications for improving crop productivity and sustainability.

I am continuing my research on the use of multi-omics approaches based on mass spectrometry techniques as part of the NCN OPUS project no 2022/45/B/NZ9/03572 entitled "Description of key mechanisms of coordination and prioritization of barley response to simultaneous biotic and abiotic stresses - multiomic approach". The current project aims to identify molecular components characteristic of trade-off mechanisms between plant responses to biotic and abiotic stresses. By subjecting barley plants to drought, *Fusarium* infection, and a combination of both stressors, I will compare the responses of the plants to gain a deeper understanding of how the plant prioritizes different stress responses.

Studying two cultivars with extremely different resistance to drought will also provide valuable information about the similarities and differences in the combined stress response in barley. This will help identify key genes and pathways involved in the plant's response to these stressors, ultimately leading to the development of more resilient crop varieties.

Overall, this project has the potential to enhance our understanding of how plants respond to multiple stressors and could have practical applications in developing more resilient crops to ensure food security in the face of changing environmental conditions.

References

5. OTHER SCIENTIFIC AND RESEARCH ACHIEVEMENTS - Presentation of significant scientific or artistic activity carried out at more than one university, scientific or cultural institution, especially at foreign institutions

I specialize in utilizing metabolomic techniques in plant biochemical projects. My research involves the use of liquid chromatography with a photodiode array detector as well as liquid chromatography combined with mass spectrometry to investigate complex relationships at the molecular and physiological levels in plant interactions with the environment, which are of interest to systems biology. An important aspect of my work is the development and

optimization of methodologies for mass spectrometry analysis, including LC-MS/MS and LC-MSn, on various plant materials such as tissues, *in vitro* cultures, and root exudates. This broad analytical expertise enables me to conduct interdisciplinary research projects spanning biology, chemistry, and phytotherapy.

Furthermore, I have collaborated with research groups from Poland and abroad, resulting in scientific publications (their number is given in parentheses). These collaborations will be discussed in detail in the following subsections (5.1 and 5.2):

•2017-2023: prof. dr hab. Monika Rakoczy-Trojanowska, Department of Genetics, Plant Breeding and Biotechnology, Warsaw University of Life Sciences, Warszawa, Poland (1)

• 2020-2022: dr. Stéphane Hacquard research team, Cluster of Excellence on Plant Sciences, Max Planck Institute for Plant Breeding Research, Cologne, Germany (1);

• 2017-2022: dr. Kenichi Tsuda research team, Department of Plant-Microbe Interactions, Max Planck Institute for Plant Breeding Research, Cologne, Germany (1);

• 2013-2020 prof. dr hab. Przemysław Ł. Mikołajczak Department of Pharmacology, Poznań University of Medical Sciences, Poznań, Poland (3)

• 2013 prof. Przemysław M. Mrozikiewicz research team, Laboratory of Experimental Pharmacogenetics, Department of Clinical Pharmacy and Biopharmacy, Poznan University of Medical Sciences, Poznań, Poland (1)

• 2013-2020 dr hab. Marcin Ożarowski, Department of Biotechnology, Institute of Natural Fibres and Medicinal Plants, Poznań, Poland (9)

• 2013-2015 prof. dr hab. Paweł Bendarek, research team, Department of Biochemistry of Natural Products, Institute of Bioorganic Chemistry PAS, Poznań, Poland (1)

• 2010-1017 prof. dr hab. Iwona Szarejko research team, University of Silesia, Faculty of Biology and Environmental Protection, Department of Genetics, Katowice, Poland (1)

•2010-2015 prof. dr hab. Maciej Stobiecki research team, Department of Biochemistry of Natural Products, Institute of Bioorganic Chemistry PAS, Poznań, Poland (2)

• 2010-2013: dr. Juana Francisco Zamora-Natera's research team, University of Guadalajara, Mexico (2)

5.1. Prior to the award of the Ph.D. degree

During my PhD, I collaborated with other research groups to develop my skills related to mass spectra interpretation and metabolomics data processing. For example, I worked with a team led by Prof. Maciej Stobiecki from the Institute of Bioorganic Chemistry, Polish

Academy of Sciences together with dr. Juana Francisco Zamora-Natera's research team, University of Guadalajara, Mexico, on their study of the metabolite profiling of Mexican lupines. The studies, published in Phytochemistry as well as the Journal of Natural Products in 2013 and 2010, respectively, analyzed flavonoid glycoconjugates from 12 lupine species using two LC/MS systems. Using CID/MS^n experiments, we identified 175 flavonoid glycoconjugates and characterized their structures by identifying the aglycones, type of sugar moieties, and acyl substituents through collision-induced dissociation experiments and MS^n spectra interpretation. The information obtained from the flavonoid conjugate profiling was used to compare the chemotaxonomy of the studied lupine species, leading to a clear-cut discrimination between North American and Mediterranean lupines.

I also participated in a study on the effect of rosemary specialized metabolites on memory in model animals as part of a research project led by Prof. Przemyslaw M. Mrozikiewicz from the Institute of Natural Fibres and Medicinal Plants and the Department of Clinical Pharmacy and Biopharmacy at Poznan University of Medical Sciences. The project, funded by the Polish Ministry of Science and Higher Education and published in Fitoterapia in 2013, evaluated the effects of subchronic administration of leaf extract from *Rosmarinus officinalis* L. on the behavioral and cognitive responses of rats linked with acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) activity in the hippocampus and frontal cortex. My research task within the project was to profile the specialized metabolites in the leaf extract. The results suggested that *R. officinalis* led to improved long-term memory in rats, which can be partially explained by its inhibition of AChE activity in the rat brain. Thus, the study suggests that *R. officinalis* can be a valuable proposal for the prevention and treatment of dementia. The high quality of my research in the field of metabolomics of phenolic specialized metabolites is reflected in my publications in international journals from the JCR list with a total impact factor of 9.513 and 215 citations, published between 2010 and 2013.

5.2. After the award of the Ph.D. degree

5.2.1. Techniques for structural characterisation of phenolic compounds

During my Ph.D. studies at the Institute of Plant Genetics of the Polish Academy of Sciences in Poznań, specifically at the Department of Pathogen Genetics and Plant Resistance, Metabolomic Team, under the supervision of Professor Piotr Kachlicki, my research was focused on the Polapgen-BD project (WND-POIG.01.03.01-00-101/08, POIG) which aimed to identify molecular markers of drought resistance. The results of my PhD work were published

in (Piasecka, et al. 2015 Journal of Mass Spectrometry; 50 (3): 513-532). I conducted a study that focused on profiling and structural analysis of phenolic secondary metabolites in methanolic extracts obtained from the leaves of nine different barley varieties. I developed and employed four different analytical approaches to obtain complementary structural information about the phenolic metabolome of barley. A low-resolution mass spectrometer equipped with an ion trap analyzer provided detailed information about the structures of 165 metabolites of barley, based on the fragmentation of compounds. The study's value was highlighted by the use of two ionization modes that provided complementary information on glycosides and acylated glycosides of flavones, acylated polyamines, and hydroxycinnamic acid derivatives. Additionally, fragmentation of hordatines, flavones acylated directly on the aglycone, isomers of chlorogenic acids, and hydroxyferulic acid derivatives were described for the first time. The results of compound identification were further confirmed through high-resolution mass spectra analysis using a tandem instrument with a time-of-flight detector. Using NMR analyses, the structures of five metabolites, including three that were previously unknown in barley, were established. Preparative chromatography procedures were also developed for the isolation and purification of selected metabolites from barley leaves. The use of a two-step separation with two types of column packing materials, polyamide and RP C18, significantly improved the separation efficiency and allowed for the extended identification of 109 metabolites present in trace amounts. The method of two-step separation of metabolites was the subject of a patent application. Among the study, a wide range of structures hitherto unknown in barley was identified, including such compounds as 6-*C*-(6"-*O*-glucosyl)-glycosides of flavones, polyamines acylated with a few hydroxycinnamic acids, and chlorogenic acid isomers. Profiling of phenolic secondary metabolites was also used for the chemotaxonomic classification of nine varieties of barley from different geographical regions and to find metabolomic markers that differentiated varieties under drought conditions.

The research skills I acquired during my metabolomic study, specifically mass spectra interpretation, were summarized in a review article by (Kachlicki et al. in 2016. Molecules, 21(11): 1494). The review article discussed the application of mass spectrometry in the structural characterization of flavonoid glycoconjugates. Various mass spectrometric techniques, with different ionization systems, molecule fragmentation, and metal ion addition, were explored for improving the structural characterization of natural products. The review presented several strategies for characterizing positional isomers and isobaric compounds in flavonoid glycoconjugates and their derivatives. The use of ion trap mass spectrometers with

MS^n allowed for multiple fragmentation steps, providing additional information about the structural dependencies of molecules. This is especially crucial for the analysis of flavonoids and their derivatives, where it may be necessary to characterize aglycones that occur in different combinations. For the identification of aglycones in flavonoid conjugates, at least MS3 analysis is necessary, which can be accomplished through either in-source fragmentation or consecutive fragmentation in ion trap mass spectrometers. These methods can distinguish positional isomers of aglycones with different hydroxylation patterns.

5.2.2. Metabolomics in phytopharmacological studies on neurodegenerative disorders

I continued successful cooperation with Poznań University of Medical Sciences and the Institute of Natural Fibres and Medicinal Plants to study metabolite profiling in plants with phytopharmacological potential. During this research, I utilized two complementary analytical methods, LC-MS/MS and LC-MS^n, supported by fast screening with UPLC-PDA for metabolite profiling. My cooperation with the research team of Prof. Przemysław Ł. Mikołajczak from the Department of Pharmacology, Poznań, Poland, provided the opportunity to discuss the global trend of using natural sources to discover and develop new drugs to prevent or slow the progression of neurodegenerative diseases such as Alzheimer's and Parkinson's diseases. My contribution to this collaboration relied on identifying biologically active compounds from plant extracts. The metabolite profiling of various plant families expanded my experience in interpreting mass spectra.

A publication (Ożarowski et al. 2015; Evidence-Based Complementary and Alternative Medicine, Article ID 145140], highlighted the potential of AChE and b BuChE enzyme coinhibition as a treatment option for Alzheimer's disease. The article examined the effect of subchronic administration of a 70% ethanol extract of *Eryngium planum* L. roots on behavioral and cognitive responses in rats, as well as the expression levels and activities of both enzymes, and beta-secretase (BACE-1) in the hippocampus and frontal cortex of rat tissues. The results showed that *E. planum* improved long-term memory in rats, regardless of the presence of scopolamine. The study suggests that extract from *E. planum* may be a promising candidate for the treatment of neurodegenerative diseases, and its mechanism of action is likely complicated, involving various phytochemicals found in the extract. I identified 64 predominant metabolites in the extracts, including triterpenoid saponins, phenolic acids, polyphenolic acids, and others. *p*-Coumaric, caffeic, and ferulic acids were identified in conjugation with glycosides, as well as four glycosides and one glucuronide of rosmarinic acid, were identified. The main

constituents of the *E. planum* extract were found to be triterpenoid saponins with barrigenol skeleton. The study suggests that *E. planum* L. could be a potential source of new therapeutic medicines for the treatment of dementia and Alzheimer's disease.

In collaboration with Prof. P. Ł. Mikołajczak, I conducted research on the effects of subchronic administration of *Melissa officinalis* extract on behavioral and cognitive responses in a scopolamine-induced model of rats (Ożarowski et al., 2016, Evidence-Based Complementary and Alternative Medicine, Article ID 9729818). The study investigated the levels of AChE, BuChE, and BACE-1 mRNA and AChE and BuChE activities in the hippocampus and frontal cortex of rats. Our findings showed that *M. officinalis* extract significantly decreased AChE mRNA levels by 52% in the cortex and inhibited BACE1 mRNA transcription in both the frontal cortex and hippocampus of rats, indicating that *M. officinalis* extracts may modulate beta-secretase activity. My contribution to this project was focused on identifying metabolites in the plant extracts. The most common compounds observed in the analyzed samples of *M. officinalis* were bioactive caffeic acid esters and flavone glycosides, with rosmarinic and lithospermic acids being the main types of phenylpropanoids. The study also revealed the presence of hydroxyjasmonic acid and its derivatives, teucrol, and sagecoumarin in the Melissa genus for the first time, while luteolin and apigenin glycoconjugates were already known phytochemicals in this species.

Another successful project conducted in cooperation with Prof. Mikołajczak involved the examination of the effects of *Salvia miltiorrhiza* root extract on memory and cholinesterase activity in rats (Ożarowski et al., 2017, Physiology and Behavior, 173: 223-230). *S. miltiorrhiza* has been traditionally used in Asian medicine to treat cardiovascular and central nervous system disorders. Our findings indicated that *S. miltiorrhiza* extract inhibited AChE activity in the hippocampus and frontal cortex and caused a decrease in beta-secretase transcript levels, suggesting a potential role in alleviating symptoms of Alzheimer's disease.

5.2.3. Metabolomics in searching for new phytopharmaceuticals

As part of my collaboration with Prof. Marcin Ożarowski and Prof. Agnieszka Seremak-Mrozikiewicz from the Institute of Natural Fibres and Medicinal Plants, we evaluated the phytopharmacological potential of closely related *Salvia* species, *S. miltiorrhiza* and *S. przewalskii* [Ożarowski et al. 2017, Phytochemistry Letters 20: 331-338]. My role in the project was to determine the metabolite profile present in both *Salvia* species using two complementary LC-MS methods. We identified a total of 39 metabolites, grouped into phenylpropanoids and

diterpenes. The polyphenolics were further classified as monomers, dimers, trimers, and tetramers of hydroxycinnamic acids, with only two metabolites not being derivatives of caffeic acid. The diterpenes tanshinones were mainly from two skeleton forms: 20-norabietanes and 19,20-dinorabietanes. Phenylpropanoids were present at a higher content than terpenoids in both studied species. We tentatively identified seven metabolites for the first time, such as rosmarinic acid di-glucoside, salvianolic acid B glucoside, and didehydrosalvianolic acid B. The dominant metabolites were caffeic acid tetramer, lithospermic acid B, salvianolic acids, and cryptotanshinone in both *Salvia* species, which were postulated as potential phytopharmaceuticals with strong antiradical scavenger activity.

In another study, published in Acta Poloniae Pharmaceutica - Drug Research [Hadas et al. 2017; 74921-928], we investigated the impact of extracts from three *Passiflora* species on chronic progressive diseases of the central nervous system caused by free-living amoebae. Effective treatment of this disease is lacking due to the amoebae's encystation, making them highly resistant to anti-amoebic drugs. We evaluated alcoholic extracts from leaves of *P. incarnata, P. caerulea*, and *P. alata*, as well as from callus cultures for their amoebicidal activity. Phytochemical analysis showed that all extracts contained mainly phenylpropanoic and flavonoids. The biological study revealed that all extracts showed amoebostatic and amoebicidal properties. Extracts of *P. alata* leaf and callus showed the most effective activities, which correlated with the highest concentration of total phenolics and flavonoids compared to other extracts.

The dry crude extracts from *Passiflora* species were evaluated for their anti-leukemic activity using similar plant material [Ożarowski et al. 2018. Brazilian Journal of Pharmacognosy, 28: 179-191]. The extracts were tested on two human acute lymphoblastic leukemia cell lines, and their phytochemical composition was assessed using two systems of LC-MS for the leaves of three *Passiflora* species. The extracts of *P. alata* and *P. incarnata* demonstrated potent inhibitory activity against human acute lymphoblastic leukemia CCRF-CEM, while *P. caerulea* showed little or no activity. The differences in the quantity of chemical compounds could determine the various pharmacological potency of the extracts. The crude extract from *P. alata* leaves was suggested as a complementary therapy for cancer patients.

In a model of chronic bronchitis in rats, I conducted a metabolomic analysis on extracts of *Platycodon grandiflorum* [Buchwald et al. 2020, Molecules: 25 (21)]. The extract showed promise as a treatment option for chronic bronchitis. *P. grandiflorum* is widely used in traditional Asian medicine as a remedy for respiratory diseases. I detected and identified

differences in saponin and inulin levels in water extracts of roots and callus. Chronic bronchitis was induced by sodium metabisulfite, and the animals were treated with the extracts for three weeks. The level of bronchitis genetic markers was determined in bronchoalveolar lavage fluid, while the CRP level was measured in serum. The extracts of *P. grandiflorum* mostly reversed this phenomenon, leading to control values. Therefore, a strong anti-inflammatory effect of the extracts from *P. grandiflorum* was suggested.

I have initiated a new collaboration with Dr. Agnieszka Gryszczyńska's team from the Institute of Natural Fibres and Medicinal Plants. Our collaborative research focuses on the pharmacological effects on metabolites of *Chamaenerion angustifolium* (L.) (*Epilobium augustifolium*) herb. My contribution to this project involves metabolite profiling and the determination of the concentration of bioactive compounds in different tissues and developmental stages of the plants. In [Gryszczyńska et al. 2018, Industrial Crops and Products, 123: 208–220] we compared two *ex vitro* lines, and harvested the herb during and after the flowering period. I utilized two complementary LC-MS systems and LC-PDA approaches to conduct qualitative and quantitative analyses of the herb metabolite profiles. I identified 45 phenolic metabolites, including gallic acid, oenothein B, and quercetin 3-*O*-arabinoside, as the principal compounds. The highest accumulation of oenothein B, β-sitosterol, and ellagic acid was found in both lines, while the total polyphenols content (expressed as gallic acid equivqlent) was predominant in the LC-PDA. The results showed that the flowering period was a better source of active compounds. The *ex vitro* cultivation method produced plants of *Ch. angustifolium* with a chemical profile similar to that of field crop plants, confirming the application potential of this method. Additionally, I underwent an internship with Dr. Gryszczyńska's research team to receive training on antioxidant analysis using multiple methods, such as scavenging ability measured by the DPPH radical assay and the total antioxidant potential of the extracts determination by the ferric reducing/antioxidant power assay (FRAP).

As part of our joint research efforts with the Institute of Natural Fibres and Medicinal Plants between 2013 and 2020, we have published nine articles in JCR-listed journals, with a total impact factor of 20.783 and a citation count of 282.

5.2.4. Metabolomics in searching for plant-microbe interactions

After beginning my work as a postdoc in the Department of Plant Functional Metabolomics at IBCh PAS, I joined a collaboration with the Department of Plant Microbe

Interactions at the Max Planck Institute for Plant Breeding Research in Cologne, focusing on metabolomic analysis of plant-microbe interactions. The complex relationship between plants and the microorganisms that colonize their roots, or microbiota, depends heavily on plant metabolites. While plants have evolved a complex immune system that can detect and respond to microbial invasion, it is unclear how this system accommodates beneficial microbes in roots and maintains a healthy host-microbe balance. In our publication [Wolińska et al. 2021, Proceedings of the National Academy of Sciences of the United States of America, 118(49):e2111521118], we discussed dysbiosis, which is characterized by an imbalance in the microbiota that negatively affects plant health.

I presented changes in the accumulation of tryptophan derivatives, including indole glucosinolates, along with their hydrolysis products, camalexin and indole-3-carboxylic acids, in different *A. thaliana* mutants grown with a multikingdom synthetic microbial community. Our results suggest that the plant immune system, specifically Trp-derived compounds, plays a crucial role in controlling fungal load in plant roots, helping to prevent dysbiosis and maintain the growth-promoting effects of the root microbiota. The study also highlights the importance of both host- and microbe-encoded mechanisms in maintaining a healthy host-microbe relationship in roots where bacteria and fungi coexist. Additionally, the study shows that bacterial commensals and host tryptophan metabolism are both necessary to control fungal load and promote plant growth and survival.

This topic was continued in (Basak, Piasecka et al. *New Phytol*. 2023; 241(1): 329-342). This publication discusses the impact of endoplasmic reticulum (ER) bodies and tryptophan (Trp)-derived specialized metabolites on root microbiota in *A. thaliana.* ER bodies are structures in plant roots that contain PYK10 myrosinase, an enzyme that hydrolyzes indole glucosinolates (IGs), which are crucial for root–microbe interactions. The study utilized various mutants of *A. thaliana* that are deficient in ER bodies, ER body-resident myrosinases, IG biosynthesis, and Trp-specialized metabolism. Mutants showed altered bacterial and fungal communities in the rhizoplane (root surface) and endosphere (internal root tissues). The study suggests that ER bodies and their resident myrosinases modulate the profile of root-secreted metabolites, influencing root–microbiota interactions. The ER body and Trp metabolism mutants showed a partly similar impact on the root-associated microbiota structure. Overall, the data indicate that ER body-resident myrosinases and Trp metabolism are important contributors to root microbial community assembly, affecting distinct but overlapping sets of microbes. Our findings emphasize the importance of plant-microbe interactions in maintaining

plant health and suggest potential strategies for improving plant growth and resilience through microbiota manipulation.

I started collaboration with prof. Monika Rakoczy-Trojanowska from Warsaw University of Life Sciences in term of NCN grant no. 2018/31/B/NZ9/00439 “Identification, characterization and mapping of common rye (*Secale cereale* L.) genes associated with resistance to brown rust caused by *Puccinia recondita* f. sp. *Secalis*". Two strains of *P. recondita* with compatible and incompatible type of inoculation were studied in three inbred lines of rye (Krępski et al. 2024, BMC Plant Biology, 24 (1):107). An RNA-seq analysis was conducted and correlated with metabolomic data. A total of 877 unique differentially expressed genes (DEGs) were identified, most of which were up-regulated. The DEGs encoded several proteins, including cytochrome P450, receptor-like kinases, methylesterases, pathogenesis-related protein-1, xyloglucan endotransglucosylases/hydrolases, and peroxidases. The metabolomic response was highly conserved among the genotypes. Comparison of metabolomic profile after infection with compatible and incompatible isolates we concluded that the secondary metabolome of rye is altered more by the recognition of the pathogen than by a successful infection. The enrichment analysis of the DEGs and differentially accumulated metabolites (DAMs) reflected the involvement of phenylpropanoid and diterpenoid biosynthesis as well as thiamine metabolism in the rye immune response. The most differentiated metabolites of both type of isolates were benzoic acid derivatives and flavonoids (such as chrysin) and peptides (such as isoleucylglutamine, isoleucylvaline and S-adenosyl-homocysteine). Numerous immune response-related DEGs and DAMs were identified, clarifying the mechanisms underlying the rye response to *P. recondita* during the early stages of brown rust development. The correlation of DAMs and DEGs elucidated the interaction between pathways mainly related to the modulation of reactive oxygen species (ROS) levels. Specific metabolic pathways were activated in response to infection, including “Flavone and flavonol biosynthesis”, “Ascorbate and aldarate metabolism”, and “Glutathione metabolism”. Disturbances in “Starch and sucrose metabolism” were observed, likely due to disruptions in sugar homeostasis caused by the pathogen. The enrichment of certain pathways, such as “Cutin, suberine and wax biosynthesis”, suggests a reinforcement of the extracellular barrier during pathogen infections. The study supports previous findings that biotrophic pathogens significantly disrupt host carbohydrate and nucleotide sugar metabolism.

5.2.5. Evolution of secondary metabolites biosynthesis in plants

Plant secondary metabolites play critical roles in interactions between plants and other organisms, including plant innate immunity. The review, (Piasecka et al. *New Phytologist* 2015; 206 (3): 948-964) summarized the current knowledge regarding the role of specialized metabolites in plant immunity. All plant species are capable of producing natural chemicals with defensive properties, indicating that this ability has been conserved throughout evolution. Phytoanticipins, which include compounds such as alkaloids, flavonoids, and glucosinolates, are produced continuously in various plant tissues and can have multiple functions, including antimicrobial activity. In contrast, phytoalexins are induced by pathogen attack and include a wide range of structurally diverse compounds, such as terpenoids, stilbenes, and phenolics. These compounds are often produced in response to specific pathogens or pathogen strains, and their production is typically tightly regulated at the transcriptional level. Some plant secondary metabolites are only found in specific groups of plants, such as a particular family or genus, indicating that the evolution of these pathways has occurred rapidly and is unique to those lineages.

The study by (Czerniawski et al. *Plant Cell Physiol*. 2023, 16;64(1):80-93) aimed to enhance our understanding of the evolution of glucosinolate biosynthesis in species of the *Camelineae* tribe, including the *Capsella* and *Camelina* genera, which have a reduced capacity to produce and metabolize indolic glucosinolates (IGs). We used comparative metabolomics in conjunction with genetic analyses to show a weakened conservation of glucosinolate-related myeloblastosis (MYB) transcription factors, including loss of functional MYB34 protein, in these species. The introduction of functional MYB34 from *A. thaliana* partially restored IG biosynthesis in *C. rubella*, indicating that the loss of this transcription factor contributes to the backward evolution of this metabolic pathway. Furthermore, the study identifies the unique function of MYB34 in feedback regulation of IG biosynthesis, driving auxin overproduction, metabolic dysregulation, and growth retardation caused by mutations in IG biosynthetic genes. These results provide insight into the evolution of specialized metabolites in the Brassicales order.

6. Presentation of teaching and organizational achievements as well as achievements in the popularization of science

6.1. Teaching Achievements

1. June/2024: Lectures, 5 hours, to the Doctoral Program of the University of Rzeszow as part of the project: "Workshop with an Expert" on metabolomic analyses
2. July/2023 and July/2021 Conducted workshops and trainings on Metabolomic data processing during the Summer School of Bioinformatics organized by Adam Mickiewicz University, Poznan, Poland
3. Periodically 2020-up to date conducted workshops and training on Metabolomic data processing organized by Xenstats sp. z o.o. at basic and advanced level of bioinformatic analysis: 'Metabolomic data analysis - chromatography - mass spectrometry (2-day online workshop): and 'Advanced metabolomic data analysis - chromatography - mass spectrometry (2-day online workshop)';
4. Periodically 2013-2021: conducted lecturers regarding mass spectrometry analyses for Postgraduate Studies in Chemical Analytics, Faculty of Chemistry, University of Adam Mickiewicz

6.2. Scientific supervisor in diploma theses and student internships:

1. July-September/2018: direct scientific supervisor and internship supervisor at the Institute of Plant Genetics, Polish Academy of Sciences for Wojciech Witek, student at Adam Mickiewicz University in Poznań;
2. 2016-2021: direct research tutor and auxiliary supervisor of doctoral thesis of PhD student Paweł Czerniawski, Institute of Bioorganic Chemistry PAS,
   Published articles:
   Czerniawski et al. 2021, Phytochemistry; 181:112571;
   Czerniawski et al. 2023, Plant Cell Physiol., 16;64(1):80-93;
3. 2022- (in progress): direct research tutor and auxiliary supervisor of doctoral thesis.PhD student Marta Kańczurzewska, Institute of Mathematics, Poznan University of Technology
   Published articles:
   Piasecka et al. 2022, Cells; *11*(20): 3213,
   Krępski et al. 2024, BMC Plant Biology, 24:107;
4. 2023- (in progress): direct research tutor and auxiliary supervisor of doctoral thesis PhD student Mikołaj Małecki, Institute of Bioorganic Chemistry PAS

6.3. Popularization of science

I am particularly actively involved in organizing and conducting scientific workshops for preschool and school children. I have organized workshops covering simple and safe experiments in the field of biology and chemistry. I conducted workshop on request of pedagogue of schools, or as part of educational events. The list of documented events:
04/2023 workshops for the 6. class of Primary School no 19 from Poznan;
12/2016 and 11/2021 workshops for the preschool class in a public kindergarten in Biedrusko;
10/2022 and 02/2023 workshops for two of 1. class of Primary School in Biedrusko;
06/2022 workshop for 5. class of Primary School in Biedrusko;
04/2022 and 05/2015 workshops for 3. class of Primary School in Niechcice in project "To(działa)My! funded by Santander Foundation;
01/2019 organization and conducting workshops as part of the "Biologists' Night" at Institute of Bioorganic Chemistry PAS;
06/2018 workshop for Club for Children and Teenagers "Ciao" in Biedrusko;
05/2014 organization and conducting workshop as part of "The Fascinating Plant World" at Institute of Plant Genetics PAS;
04/2014 workshop for the preschool class in a Leonardo kindergarten run by the Familijny Poznan Foundation;
01/2014 organization and conducting workshop as part of "the Biologists' Night" at Institute of Plant Genetics PAS.

6.4. Research project management

- **03/2016-03/2020: Principal investigator** in National Science Center (NCN) research project SONATA, No. 2015/17/D/NZ9/03347

Project entitled: "Metabolomic and proteomic aspects of conservative plant responses in the *Poaceae* family to *Fusarium* head blight"
Publications: **H4, H5, H6**

- **04/2023 – in progress: Principal investigator** in National Science Center (NCN) research project OPUS, No. 2022/45/B/NZ9/03572

Project entitled "Description of key mechanisms of coordination and prioritization of barley response to simultaneous biotic and abiotic stresses - multiomic approach".

6.5. Membership in the organizing committees of international conferences

Metabolomics Society, member from 2010 to 2015.

Polish Society of Mass Spectrometry from 2013 to 2018.

6.6. Other professional career information

Detailed information related to my scientific activity, including:

- a full list of articles published in scientific journals (26), and conference presentations (16 as the author of the presentation);
- information on participation in research projects as a team member-researcher (10);
- list of training and workshops held in Poland and abroad (4);
- information on peer-reviews of scientific papers (16);
- information on participation in European programs (2);
- awards and distinctions (1);
- list of filed patent applications (2);are included in Appendix 11. List of achievements.

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